"The ties that bind": Investigating the interaction between Interlocus and Intralocus Sexual Conflict using *Drosophila melanogaster*

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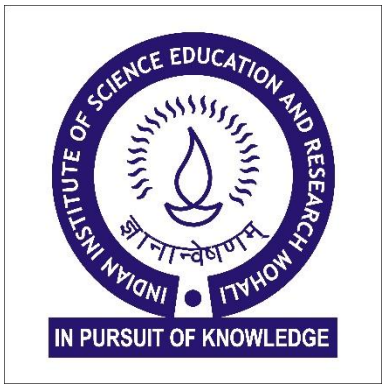
A thesis submitted for the partial fulfilment of the degree of Doctor of Philosophy



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Dedicated

to

Aaji

(the strongest,

the kindest,

and the most beautiful

human being I have known)

Declaration

The work presented in this thesis has been carried out by me under the guidance of **Prof. N. G. Prasad** at the Indian Institute of Science Education and Research Mohali. This work has not been submitted in part or in full for a degree, a diploma, or a fellowship to any other university or institute. Whenever contributions of others are involved, every effort is made to indicate this clearly, with due acknowledgement of collaborative research and discussions. This thesis is a bona fide record of original work done by me and all sources listed within have been detailed in the bibliography.

Manas Arun Samant

In my capacity as the supervisor of the candidate's thesis work, I certify that the above statements by the candidate are true to the best of my knowledge.

Prof. N. G. Prasad

Acknowledgements

Long before I had written even a single word of my PhD thesis, I had an epiphany, that writing the Acknowledgements section of one's PhD thesis is not too dissimilar to writing one's Last Will and Testament. The resemblances are uncanny. Both are written at the end of long, arduous journeys. The writer can also be assured that both are among the few samples of their writing that would be read by more than a just few pairs of eyes. Jokes apart, here's my attempt at acknowledging all the people who have played a role, both small and large, in a journey that I have invested copious amounts of sweat and tears in.

Right at the outset, I would like to offer my sincere thanks to the People of India, whose surplus labour funded the research outlined in this thesis and provided me with my fellowship. Several agencies of the Government of India acted as the conduits of this funding. I owe a great deal to the officers and the staff at the Council for Scientific and Industrial Research (CSIR) who worked tirelessly to ensure that scholarships were disbursed under trying circumstances, even in the midst of the harshest COVID induced lockdown in the world. As far as encumbrances in the disbursal of monthly scholarships were concerned, I cannot stress enough, the role played by Mr. Anuj, the Assistant to the Office of the Dean (Research and Development), who, on multiple occasions, went beyond the line of his duty to ensure that stalled fellowships were resumed. Thanks are also in order to Neena Ma'am, the Assistant to the Office of the Dean (Students), for her invaluable help in various bureaucratic challenges over the years. I must acknowledge the IISER Mohali library for all the books I issued (but did not read!), and also for the, largely, seamless access I enjoyed to various scientific journals. I am indebted to valuable comments by the reviewers, as a result of which this thesis underwent substantial improvement.

I would like to thank the members of my thesis advisory committee (Prof. N. G. Prasad, Prof. Sudeshna Sinha, and Dr. Rhitoban Raychoudhury) for their support during my PhD. I must mention the mentorship and guidance I received from Prof. Sinha in the early stages of my PhD, without which I would not have been able to attempt the mathematical modelling project outlined in Chapter 6. I would like to thank Dr. Manjari Jain, for generously letting me use the microscope facility in her lab, and also for her support during various other academic endeavours. I thank Prof. Adam Chippindale, who provided our lab

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When I started my PhD in August 2016, I, along with other students who joined around the same time, inherited a fully-established, state of the art, ready-made research facility, the credit of which must go to the "founding" PhD students of the lab: Bodhi, Vinesh, Vanika, and Karan Singh. These four ridiculously talented, and immensely hardworking researchers established, re-established, and re-re-established the lab every time it moved to a new building - from scratch. Even more importantly, they worked out and standardised most of the experimental protocols described in these pages. Without their numerous contributions, my work would have been infinitely more difficult. My association with these four goes back to the summer of 2011, when I joined the lab as a summer intern, my first exposure to "real" scientific research. As an undergraduate student with no idea of what research was all about, I couldn't have hoped for a better environment. Bodhi, Vanika, Vinesh and, of course, NGP, created a lab culture that was warm, welcoming, egalitarian, light-hearted, but at the same time, scientifically rigorous. The experiences I had over those two months, particularly, the late-night scientific discussions over coffee with Bodhi, and of course, all those experiments I ran with my fellow summer interns (Sharmi, Pratip, Tj, Zeeshan and Niveda), left a lasting impression on me, and played a vital role in shaping who I am today as a researcher.

None of the experiments described in this thesis would have been designed or actually executed without the help and support of the PhD students whose tenures overlapped with mine: Zeeshan, Aparajita, Komal, Neetika, Tj, Kapila, Ruchika, and Akb. I would particularly like to thank someone I have always looked up to, Zeeshan, for his counsel (both academic and otherwise) at various points in time, and for giving me the jersey of a football club that I am loath to associate myself with now; Kapila, for his notoriously

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Over the years, I have had the good fortune of getting to know, working with and learning from a large number of master's students who were associated with the lab. Many of these individuals actively participated in the design and execution of the experiments described in this thesis. At the risk of inadvertently omitting some names (apologies to those who are missing!!), I would like to individually thank Abhilasha, Anshu, Mehreen, Lokesh, Vrinda, Sharmi, Tj, Radhika, Shady, Ruchika, Athira, Martik, Megha, Ekta, Akanksha (Bells), Ateesha, Sushma, Prakhar, Bhatti, Aatashi, Harsha, Reshma, Vaibhvi, Chinmay, Teju, Bisu, Nitin, Neeraj Meena, Amisha, Abhishek, NS, Jigisha, Sohit, Santy, Imran, Akshay (Patta), Paresh, Broti, Soumyadip, Manki, Priya Bhatt, Kimaya, Vandana, Mubarak, Rakesh, Srishti, Shradha, Ruchira, Anjali, Shreya, Shivanshu, Diksha, Ajinkya, Rohit, Somesh, Sudeep, Arya, Sanket, Akanksha and Koyna for making the lab such an extremely fun environment to be in.

I would specially like to mention Sharmi, Tj, Shady and Radhika, for having been the best peers one could have hoped for, while beginning one's first independent research project. Special thanks are also due to Abhishek and Jigisha, who were simply the best co-workers among all the people I have worked with so far. I also had the good fortune of mentoring a group of extremely talented people, including Rakesh, Srishti, Shradha, Ruchira, Priya Bhatt, Akanksha, and Koyna. While it is not entirely clear whether I was a good mentor to them, I certainly ended up learning a lot in these interactions, and I must thank each of them for that. I must specially mention Rakes Bhai for the sheer tenacity, perseverance and hard work he has demonstrated in pursuit of his goals and dreams, and I would like to assure him that it's only a matter of time before he lands an amazing PhD position. I would also

like to thank Anjali, an enviably gifted researcher, for her help while analysing the wing shape data (Chapter 5b).

Some of the people in the long list of names above have continued to remain good friends with me, despite the fact that they have now flown to distant shores. Among them are Martik, who gave me the best present I have yet received; Amisha, who undertook an enormously perilous voyage across the oceans to carry that present to India; Broti, who is among the finest storytellers I know, and whose attention to detail is only rivalled by the great Frederick Forsyth; and Bhatti, who has somehow managed to remain the same, pure, unadulterated Bhatti that walked into the lab in the summer of 2014.

I would also like to acknowledge the support I have received over the years from a large number of other individuals I call friends: Dr. Divya Sharma (who I sincerely hope forgives me for the unpardonable offence of having forgotten to include her name in an earlier draft of this essay), Chajjo (my undisputed bff, albeit self-proclaimed), Mahima (my companion for visiting sundry historical monuments in Delhi), Yogesh (who I must apologise to for not having bothered to read the copy of the Bhagwadgeeta he gifted me on one of my birthdays), Akshay (who I shall eternally remain cross with for telling me about his wedding the sum total of one day before it was supposed to happen), Aniket (who I dedicated my MS thesis to - but only because he asked), Manaoj (a physicist, a poet, a master of puns, and an absolutely delightful human being in general, unless you happen to be with him on a mountain), Vidhika (who once dragged me all the way to the Interstate Bus Terminal at 1 AM - to have chai), Shantanu (the ideal person to have by your side when your city receives 960 mm of rainfall within a span of a few hours), and Mihir (who, inexplicably, has continued to remain friends with me for more than two and a half decades). I am also thankful to Anubhav, Prashant, Tejasi, Diksha, Jasmine, Priya, Amol, Anmol, Sajan, Anushree, Debottam and Rohan, the people with whom I played Table Tennis - and in some cases, even won a few medals.

It was only a few days ago that I remarked to someone that I have made some questionable decisions in my life, but if there's one aspect of my life where I have done rather well, then it's my choice of friends, and I couldn't possibly be prouder of the fact that I am friends with Neeraj, Arul and Tj. For a good part of the last decade, the three of them have been my best friends, my closest companions and even my agony aunts on a number of occasions. I couldn't have finished this PhD without them. Special thanks to Neeraj, for

introducing me to the song that, at least partially, served as the inspiration behind the title of this thesis; Arul, for introducing me to the art of being a sceptic and impressing upon me that sometimes it is important to take a stand (or to quote Pash, *beech ka raasta nahin hota*); and Tj, for introducing me, in himself, to the finest man I know.

As we move closer to the end of this essay, I would like to tip my hat to my PhD supervisor, Prof. N.G. Prasad. I could write paragraphs about how supportive he is as a supervisor, how much freedom one gets as his student, and the lengths he goes to in order to help his students. However, I would be wasting my breath, as all these things, including the famed "friend, philosopher and guide" cliché, have already been described in great detail in past theses from our lab. Therefore, I will restrict myself to describing the two most important ways in which he has made a lasting impact on me. The first is a lecture I attended on a cold, wintry January afternoon in 2011. I vividly remember the figure drawn on the blackboard that described the relationship between genetics, developmental biology, ecology and evolutionary biology with the help of four graphs and four arrows. I remember thinking to myself, "this is the kind of stuff I would like to study." That lecture was taught by NGP. And second, whether directly or indirectly, he has been responsible for some of the happiest memories I have made over the last decade at IISER Mohali, and I will cherish these moments till my last breath.

I would also like to thank Jyothi Ma'am for constantly reminding me that I am underweight! On a serious note, and I am sure I speak for many of my lab mates when I say this, the warmth, the generosity and the affection with which both NGP and Jyothi Ma'am treated us, and counted us among their own family, made a world of difference to us, throughout the years, but particularly so, during those harrowing months of COVID in the summers of 2020 and 2021.

Finally, I would like to acknowledge the role played by my family: Mama, Mami and Tutu, for their constant support; Mayuri Tai, for always inspiring me through her achievements (scholastic and otherwise); and lastly, Aai, for everything, from raising me as a single parent, to continuously asking me to do better, and inspiring me (and at times, even shaming me) by her intense drive to constantly learn new things, including new languages, well into her sixties.

With that I would like to conclude the Last Will and Testament of my PhD. I pray that as this journey ends, and a new one begins, I meet people just as wonderful as the folks described above. Amen.

Synopsis

A divergence in the evolutionary interests of males and females leads to sexual conflict (Parker 1979). Conceptually, sexual conflict can be of two distinct kinds: Interlocus Sexual Conflict (IeSC) and Intralocus Sexual Conflict (IaSC) (Schenkel et al. 2018).

IeSC is typically modelled as a conflict over mating rates (Gavrilets et al. 2001; Rowe et al. 2005). Male fitness is assumed to increase linearly with mating rates, while females are assumed to have an intermediate optimum mating rate. Mating rates themselves are modelled as functions of traits that are sex-limited in their expression, i.e., persistence traits in males and resistance traits in females. At the same time, IeSC can be extended to other aspects of male – female reproduction related interactions such as the interaction between male ejaculate and the female reproductive tract (Sirot et al. 2015). Sexually antagonistic coevolution, a process where one sex evolves adaptations that increase the fitness of that sex, but exert a cost on the fitness of the opposite sex, triggering counteradaptations in the opposite sex, is a hallmark of IeSC. IeSC has been documented in diverse organisms including water-striders (Khila at al. 2012), spider mites (Macke et al. 2014), snails (Swart et al. 2020), and even plants (Lankinen et al. 2017).

IaSC, on the other hand, is a conflict over traits that are common to both males and females (i.e., traits with a positive intersexual genetic correlation), but have vastly different sex-specific fitness optima (Bonduriansky and Chenoweth 2009). At the level of a locus IaSC ensue when the allele that is favoured in males is different from the allele that is favoured in females (Haldane 1962). IaSC is thought to be resolved by the evolution of sex-specific genetic architecture, leading to the evolution of sexual dimorphism (Bonduriansky and Chenoweth 2009). IaSC, too, has been reported in a large number of diverse taxa, including beetles (Berger et al. 2016), mammals (Stulp et al. 2012), reptiles (Svensson et al. 2009), fish (Barson et al. 2015) and plants (Delph et al. 2011).

In their preliminary mathematical formalisms, IeSC (which deals with sex-limited traits) and IaSC (which deals with traits that are common to both males and females) are mutually exclusive phenomena. However, there have been several arguments in the favour of an interaction between the two kinds of sexual conflict. Some of these arguments revolve around the idea that the traits involved in IeSC may not be entirely sex-limited in their

effects (Pennell and Morrow 2013; Pennell 2016). For example, it is possible that resistance and persistence related traits are genetically correlated, meaning selection in one sex could have cascading effects on selection on the other sex. It is also possible that genes that code for resistance and persistence traits have pleiotropic fitness effects when expressed in the opposite sex. Alternatively, it is possible that traits involved in IaSC are under sexual selection, meaning a change in the intensity of IeSC in the population could trigger a change in the degree of sexually antagonistic selection on such traits. However, the empirical evidence for whether IaSC and IeSC interact is scarce.

In this thesis, I investigated the potential interaction between IaSC and IeSC in a laboratory population of fruit flies. *Drosophila melanogaster* is an ideal model system to investigate the interaction between IeSC and IaSC, because it has been at the forefront of research investigating both kinds of conflict (Chippindale et al. 2001; Filice and Long 2016; Long and Rice 2007; Nandy et al. 2013a). I used an experimental technique called hemiclonal analysis, which uses special genetic constructs called clone generator (CG) females (Rice 1996). CG females have a homozygous-viable translocation between chromosome II and chromosome III, as well as a compound X chromosome. This allows one to sample and clone a panel of entire haploid genomes (or hemigenomes), with the exception of the dot chromosome. These chromosomes can then be expressed in males and females carrying complementary chromosomes randomly sampled from the same source population, allowing one to measure additive genetic variances and covariances for a large number of traits.

In the first part of my thesis, I asked the following question: Does experimentally changing the intensity of IeSC affect the signal of IaSC in the population? Using the CG females, I sampled a panel of 39 hemigenomes from a laboratory adapted population of *D. melanogaster* called "LH". I measured the contribution of each hemigenome to male and female fitness at three different intensities of IeSC obtained by varying the adult sex ratio: 1:3 male biased sex ratio (strong IeSC), equal sex ratio (intermediate IeSC), and 3:1 female biased sex ratio (weak IeSC). At each sex ratio, I measured two parameters corresponding to the strength of IaSC: intersexual genetic correlation for fitness ($r_{g,w,mf,}$) and the proportion of sexually antagonistic variation. In contrast to previous similar studies (Chippindale et al. 2001; Collet et al. 2016; Innocenti and Morrow 2010; Ruzicka et al. 2019), I found that at each of the three sex ratios $r_{g,w,mf}$ was significantly greater than 0. Furthermore, $r_{g,w,mf}$ was higher at male biased and equal sex ratios, relative to the female biased sex ratio.

Correspondingly, the proportion of sexually antagonistic fitness variation was lower at the male biased and equal sex ratios, relative to the female biased sex ratio. These results suggest that experimentally increasing the intensity of IeSC led to a slight amelioration in the intensity of IaSC. However, it must be noted that differences between sex ratios were not statistically significant.

In the next two parts (part 2 and part 3) of my thesis, I investigated the potential mechanism underlying the pattern I detected in part 1 which suggested that increasing the intensity of IeSC leads to a slight amelioration of the signal of IaSC in the population. First, I measured a suite of potentially sex-limited traits for each hemigenome line, and investigated the selection acting on each trait at the three different sex ratios. Typically, male biased sex ratios are thought to lead to stronger sexual selection (Janicke and Morrow 2018). Several empirical studies have shown that evolution under male biased sex ratios leads to a rapid evolution of reproduction-related traits linked to sexual selection and IeSC (Nandy et al. 2013a; Nandy et al. 2013b; Nandy et al. 2014; Wigby and Chapman 2004). However, there is a sizable body of theoretical literature that suggests that male biased sex ratios may not always be associated with stronger sexual selection (Klug et al. 2010; Kokko et al. 2012). Therefore, as the first step, I validated that my sex ratio treatments conformed to my expectation that sexual selection and IeSC would be stronger at the male biased sex ratio. I was able to show that the additive genetic variance for relative fitness was the highest at male biased sex ratio, followed by equal sex ratio and the lowest at female biased sex ratio for both males and females. Females held at male biased sex ratio also experienced greater male induced mate-harm indicated by a sharp drop in fecundity at the male biased sex ratio. Furthermore, male reproduction related traits such as persistence related traits as well as sperm competitive ability were under stronger selection at male biased sex ratio. Having established that the sex ratio treatments indeed corresponded to variation in sexual selection along expected lines, I next investigated whether genetic correlations between persistence and resistance related traits or pleiotropic fitness effects of resistance and persistence traits in the opposite sex drove patterns of the interaction between IaSC and IeSC. Regardless of how I measured resistance and persistence, I could not detect any statistically significant genetic correlations between resistance and persistence. On the other hand, I found that traits corresponding to male persistence were positively genetically correlated with female fitness, possibly hinting at a role of pleiotropic fitness effects of genes coding for persistence when expressed in females. My data also allowed me investigate trade-offs

between male reproductive traits. However, I found *positive* genetic correlations between various male pre- and post-copulatory reproductive traits such as sperm competitive ability and mating related traits. I also found evidence to suggest that there was IeSC over copulation duration at the male biased sex ratio, with males benefiting, but females paying a fitness cost, due to longer copulations.

In the next part of my thesis, I investigated a set of traits that are shared between males and females, i.e., traits with positive intersexual genetic correlations. Strong intersexual genetic correlations constrain sex-specific adaptation; sex-specific natural and/or sexual selection has the potential to displace the opposite sex away from its sex-specific fitness optimum (Lande 1980). Given that there were differences in the strength of sexual selection between the three sex ratios, I hypothesised that the degree of sexually antagonistic selection on traits that are shared between males and females would also be different between the three sex ratios. To investigate this possibility, I first measured locomotory activity at male biased and female biased sex ratios, egg to adult development time, and dry body weight for males and females carrying each of the sampled hemigenomes. These traits are ideal to investigate the patterns of sexual antagonism for several reasons. First, all three are strongly sexually dimorphic. Second, there is strong evidence that locomotory activity drives IaSC in *D. melanogaster* (Long and Rice 2007), as well as some evidence for sexual antagonism for development time and body size (Lund-Hansen et al. 2020; Prasad et al. 2007). However, surprisingly, I could not detect any statistically significant genetic correlations between male and female locomotory activity, suggesting that this is not a trait that is shared between males and females in the population under study, and can therefore, cannot meditate patterns of IaSC. I also did not detect any statistically significant selection gradients on locomotory activity in males. However, there was a positive genetic correlation between female activity and female fitness at female biased sex ratio. Furthermore, female activity at female biased sex ratio was also genetically correlated with the antagonism index (the projection of male and female fitness scores on the axis of sexually antagonistic fitness variation). Thus, while locomotory activity was not a shared trait between males and females, female locomotory activity was still correlated with sexually antagonistic fitness variation at female biased sex ratio.

In contrast to locomotory activity, I found a strongly positive intersexual genetic correlation for dry body weight and development time. The linear selection gradients on dry body weight were not significantly different from 0 at either of the sex ratios in both sexes,

providing no evidence that dry body weight could drive signals of IaSC. On the other hand, I found that there was strong selection for faster development in both sexes at the male biased sex ratio, but not at the female biased sex ratio. All else being equal, this strong sexually concordant selection on development time at the male biased sex ratio can explain the slight amelioration in the signal of IaSC at male biased sex ratio I found in the first part of the thesis.

Next, I extended this analysis to a multivariate trait, wing shape, which is an ideal system to investigate IaSC and its interaction with sexual selection. There is ample evidence that *D. melanogaster* wing shape has substantial additive genetic variance for wing shape and that it can rapidly respond to selection (Menezes et al. 2013; Sztepanacz and Houle 2019). Wing shape is also associated with male mating success (Menezes et al. 2013; Trajković et al. 2021). Furthermore, there is evidence strong intersexual genetic correlations (Sztepanacz and Houle 2019) and sexual antagonism for wing shape (Abbott et al. 2010). With a large number of well-defined land marks on the wing surface that are conserved across *Drosophila*, wing shape is well-suited for geometric morphometric analyses. Using 11 distinct landmarks on the wing surface, I performed geometric morphometric analyses on wings dissected from males and females carrying the sampled hemigenomes. I found strong sexual dimorphism and substantial additive genetic variation for wing shape. I also detected strongly positive intersexual genetic correlations for wing shape. Interestingly, there was evidence of sexually antagonistic selection on wing shape only at male biased sex ratio, and not at the other two sex ratios. Males with shorter and stubbier wings, but females with elongated wings enjoyed fitness benefits at male biased sex ratio. This suggests that increasing the strength of IeSC and/or sexual selection led to an *increase* in the degree of sexually antagonistic selection on wing shape.

In the last part of my thesis, I investigated the resolution of IaSC using a two-locus population genetic model. While empirical research on IaSC is a few decades old, the underlying mathematical logic has been investigated by a large number of mathematical studies over the last seven decades (Bodmer 1965; Fry 2010; Haldane 1962; Kidwell et al. 1977; Owen 1953; Rice 1984). A number of theoretical studies have also investigated the resolution of IaSC by invoking several biological mechanisms including gene duplication (Connallon and Clark 2011), genomic imprinting (Day and Bonduriansky 2004), sex-specific dominance (Spencer and Priest 2016) and the evolution of sex-biased gene expression by modifier alleles (Connallon and Clark 2010). In a landmark study, Connallon

and Clark (2010) evaluated the conditions which favour the evolution of sex biased gene expression through modifier alleles on autosomes and the X chromosome. However, in their study they modelled the modifier allele to affect the fitness of the deleterious allele only, while leaving the fitness of the beneficial allele unaffected. In my thesis, I relaxed this assumption, and introduced additional parameters that controlled the effect of the modifier allele on the expression of the beneficial and the deleterious allele in one of the sexes. My results highlight that as long as the modifier allele has even the slightest effect on the expression of the beneficial allele, resolution of IaSC is not automatically guaranteed. My results also suggest that increased recombination rates may impede the resolution of IaSC, especially when selection in the sex selected for expression divergence is weak.

In conclusion, while IaSC and IeSC have been investigated in considerable detail in isolation, my thesis is among the first studies to provide empirical evidence of whether the two kinds of sexual conflict interact. My results suggest that such an interaction unfolds in complicated ways. Overall, I found a statistically non-significant trend, where increasing the intensity of IeSC led to a slight weakening of the intensity of IaSC in the population. Consistent with this trend I found that female activity was associated with sexually antagonistic genetic variation at female biased sex ratio, and there was strong sexually concordant selection on development time at male biased sex ratio, suggesting that in certain cases strengthening one kind of sexual conflict may lead to an amelioration of the other kind of sexual conflict. On the other hand, I found sexually antagonistic selection acting on wing shape, but only at male biased sex ratio, implying that at least in this case, IeSC and IaSC reinforce each other. Lastly, results from my population genetic model suggest that the resolution of IaSC via the evolution of modifiers that bring about sex-specific selection may not be as easy as previously thought.

List of Publications

1. Geeta Arun, M., Chechi, T.S., Meena, R., Bhosle, S.D. and Prasad, N.G., 2022. Investigating the interaction between inter-locus and intra-locus sexual conflict using hemiclonal analysis in *Drosophila melanogaster*. BMC Ecology and Evolution, 22, Article number: 38
2. Maggu, K., Kapse, S., Ahlawat, N., Geeta Arun, M. and Prasad, N.G., 2022. Finding love: fruit fly males evolving under higher sexual selection are inherently better at finding receptive females. Accepted for publication in Animal Behaviour, 187:15-33.
3. Geeta Arun, M., Agarwala, A., Syed, Z.A., Kashyap, M., Venkatesan, S., Chechi, T.S., Gupta, V. and Prasad, N.G., 2021 Experimental evolution reveals sex-specific dominance for surviving bacterial infection in laboratory populations of *Drosophila melanogaster*. Evolution Letters, 5(6):657-71.
4. Ahlawat, N., Geeta Arun, M., Maggu, K. and Prasad, N.G., 2021. Enemies make you stronger: Coevolution between fruit fly host and bacterial pathogen increases postinfection survivorship in the host. Ecology and Evolution, 11(14):9563-9574.
5. Maggu, K., Ahlawat, N., Geeta Arun, M., Meena, A. and Prasad, N.G., 2021. Divergence of responses to variable socio-sexual environments in laboratory populations of *Drosophila melanogaster* evolving under altered operational sex ratios. Evolution, 75(2):414-426.
6. Syed, Z.A., Gupta, V., Geeta Arun, M., Dhiman, A., Nandy, B. and Prasad, N.G., 2020. Absence of reproduction-immunity trade-off in male Drosophila melanogaster evolving under differential sexual selection. BMC Evolutionary Biology, 20(1):1-10.
7. Syed, Z.A., Chatterjee, M., Samant, M.A. and Prasad, N.G., 2017. Reproductive isolation through experimental manipulation of sexually antagonistic coevolution in *Drosophila melanogaster*. Scientific Reports, 7(1):1-8.

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Chapter 1

Introduction[1]

In a hotly debated, and much cited article that is undeniably a tour de force in modern scientific writing, Gould and Lewontin (1979) invoked Voltaire's Dr. Pangloss while describing the fan vaulted ceiling of the Tudor Chapel in Cambridge. Referring to the peculiar empty spaces between adjacent fans that are intricately ornamented with alternating motifs representing a Tudor rose and a portcullis, they wrote the following:

> "Anyone who tried to argue that the structure exists because the alternation of rose and portcullis makes so much sense in a Tudor chapel would be inviting the same ridicule that Voltaire heaped on Dr Pangloss: 'Things cannot be other than they are …Everything is made for the best purpose. Our noses were made to carry spectacles, so we have spectacles. Legs were clearly intended for breeches, and we wear them.' Yet evolutionary biologists, in their tendency to focus exclusively on immediate adaptation to local conditions, do tend to ignore architectural constraints and perform just such an inversion of explanation."

While Gould and Lewontin (1979) have been criticised on a number of occasions (Anderson 1979; Borgia 1994), they do make an important point. They intended the article as a warning against the tendency to overemphasise the role of natural selection, while ignoring other important factors such as drift, phylogenetic constraints, historical contingencies, and anatomical (or architectural) limitations. They also highlighted the frequent dissonance between selection and adaptation, a theme that is quite relevant to this thesis.

One of the simplest models of natural selection considers changes at a single locus with two different alleles. The model assumes a large randomly mating monoecious population,

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[1] Note that portions of this chapter have been published as part of a research article (Geeta Arun, M., Chechi, T.S., Meena, R., Bhosle, S.D. and Prasad, N.G., 2022. Investigating the interaction between inter-locus and intra-locus sexual conflict using hemiclonal analysis in *Drosophila melanogaster*. BMC Ecology and Evolution, 22, Article number: 38).

absence of mutation, and constant fitness of genotypes. In this model, the change is allelic frequency is given by the following identity:

$\Delta q = \frac{q(1-q)}{2\mathbf{W}} \frac{d\mathbf{W}}{dq}$ … (1) (Rice 2004), where q is the allelic frequency and **W** is the average population fitness. This identity suggests that a large, randomly mating, monoecious population is expected to evolve (in absence of mutation) along a direction where the average population fitness increases. At evolutionary equilibria, where $\Delta q=0$, the average population fitness should at least be at a local maximum, leading to "adaptation".

Quite unsurprisingly, relaxing some of the assumptions of the simplistic model described above leads to scenarios where average population fitness is *not* maximized at evolutionary equilibria. For example, if fitnesses of genotypes are not constant, but functions of genotypic frequencies, i.e., selection is frequency-dependent, the equivalent of identity (1) for such cases is the following: $\Delta q = \frac{q(1-q)}{2\mathbf{W}} [\frac{d\mathbf{W}}{dq} - E(\frac{dw}{dq})]$ … (2) (Rice 2004). This implies that when selection is frequency dependent, populations may evolve characters that do not necessarily lead to a maximization of the average population fitness, i.e., adaptation. Another example where selection may not lead to adaptation is populations with a small effective population size, such that random genetic drift has a predominant role in allele frequency change. Population genetic theory shows that in small populations, novel mutations that are beneficial are likely to go extinct as a result of genetic drift, unless selection coefficients are extremely large. Even more interestingly, when there are more than two alleles present at a locus, populations are not expected to climb to the fitness maxima via the shortest possible route (Rice 2004). When selection acts on more than one locus with epistatic interactions in fitness, the fitness maximization principle of the one locus case, even when fitnesses are constant, is violated.

Another class of phenomena that truly exemplify the constraints to adaptation by natural selection is genomic conflict, i.e., situations where there is a sharp divergence in the reproductive interests of different parts of the genome or the same part of the genome but in different contexts (Rice 2013). Genomic conflict is ubiquitous and can manifest in myriad ways. In some cases, one part of an individual's genome can gain a fitness advantage to the detriment of the fitness of another part of the same individual's genome, and potentially even at a cost to the fitness of the individual as a whole (Ågren and Clark 2018). For example, due to their matrilinear inheritance, in many monoecious plants, mitochondria have been shown to accumulate mutations that substantially reduce male

reproductive function (a phenomenon referred to as cytoplasmic sterility), and therefore also the overall individual fitness (Hanson and Bentolila 2004). In certain cases, alleles called segregation distorters (so named because they violate Mendel's Law of Segregation) can displace their alternative alleles and ensure that they get preferentially transmitted through gametes during meiosis (Lyttle 1991). In a landmark paper, Hamilton (1967) showed that a Y-linked segregation distorter can rapidly drive the population to extinction via extremely male biased sex ratios. Transposable elements, first discovered in maize (McClintock 1950), are genetic elements that can insert themselves at other locations in the genome, potentially disrupting the function of genes located at those positions leading to negative fitness effects to the individual (Hancks and Kazazian 2016). Alternatively, it is also possible that the same part of the genome has opposite consequences for the fitness of the individual when expressed in different contexts (e.g., different tissues, different ages, different environments, or even different sexes (see below)) a phenomenon termed "antagonistic pleiotropy" (Curtsinger et al. 1994), first developed by G. C. Williams in the context of ageing (Williams 1957). Under certain conditions, antagonistic pleiotropy can maintain a stable polymorphism, leading to the maximization of the average population fitness overall, but not optimal adaptation in either context individually.

A particularly interesting case of genomic conflict ensues when there exist distinct fitness optima in males and females leading to sexual conflict. Defined for the first time in 1979 (Parker 1979), the term "sexual conflict" is typically used to describe situations that are optimal for the fitness of one sex but detrimental to the fitness of the other sex (Schenkel et al. 2018). Conceptually, sexual conflict is thought to be of two kinds: Interlocus Sexual Conflict (IeSC) or Intralocus Sexual Conflict (IaSC) (Schenkel et al. 2018).

Typically, IeSC has been mathematically modeled as a conflict over mating rates, with male fitness increasing indefinitely with increasing mating rates, while females having an intermediate optimum mating rate (Gavrilets et al. 2001; Rowe et al. 2005). Mating rates are modeled as a function of male and female traits that are sex-limited in their expression (usually called "persistence" and "resistance" traits respectively). Therefore, IeSC is a conflict between a set of loci limited in their expression to males, and a different set of loci limited in their expression to females. IeSC can also be extended to other spheres of reproductive interactions between males and females; for example, the interplay between the female reproductive tract and male ejaculate components (Sirot et al. 2015), sex allocation in haplodiploid organisms (Macke et al. 2014), or even parental investment

(McNamara et al. 2014). Various forms of IeSC have been reported in diverse taxa including crickets (Sakaluk et al. 2019), beetles (McNamara et al. 2020; Wilson and Tomkins 2014), flatworms (Patlar et al. 2020), snails (Daupagne and Koene 2020; Swart et al. 2020), and even plants (Lankinen et al. 2016; Lankinen et al. 2017). An extreme example of IeSC is traumatic insemination in species of beetles, where male genitalia cause physical injury to females during copulation (Dougherty et al. 2017). This is expected to trigger counteradaptations in females aimed at reducing this male mate harm.

IaSC, on the other hand, is a consequence of males and females sharing the same gene pool while experiencing markedly different selection pressures (Schenkel et al. 2018). IaSC is usually defined for traits that have a common underlying genetic basis in males and females, but have vastly different sex-specific fitness optima (Bonduriansky and Chenoweth 2009). At the level of a locus, IaSC arises when the allele that is favoured in males is different from the one that is favoured in females (Kidwell et al. 1977). Patterns consistent with IaSC have been reported in a wide range of organisms including rainbow trout *Oncorhynchus mykiss* (Pearse et al. 2019), the bank vole (Lonn et al. 2017), the collared flycatcher (Dutoit et al. 2018), the Raspberry crazy ant *Nylanderia fulva* (Eyer et al. 2019), and even human beings (Cheng and Kirkpatrick 2016). An interesting case of IaSC occurs in Atlantic salmon, where there is evidence of IaSC over age at reproductive maturation or the time individuals spend at sea before returning to their natal streams for reproduction (Barson et al. 2015, Mobley et al. 2020). Longer age at maturity is associated with larger body size (and greater fecundity), but also a greater risk of predation. This trade-off coupled with drastically different life-history strategies employed by the sexes results in a scenario where early maturity is favoured in males but later maturity in females. Examples of sexually antagonistic selection on traits shared between males and females are numerous and include body size in humans (Stulp et al. 2012), immunocompetence in lizards (Svensson et al. 2009) and insects (Sharp and Vincent 2015; Vincent and Sharp 2014), colour patterns in birds (Price and Burley 1994), and leaf area in flowering plants (Delph et al. 2011) among others. IaSC is thought to be resolved by the evolution of sex-specific genetic architecture (Bonduriansky and Chenoweth 2009) via a variety of different mechanisms including gene duplication (Connallon and Clark 2011), genomic imprinting (Day and Bonduriansky 2004), sex-specific dominance (Spencer and Priest 2016) and sex-biased gene expression through modifiers of expression (Connallon and Clark 2010).

In their traditional formalisms, IaSC (which deals with traits that are shared between the sexes) and IeSC (which deals with traits that are sex-limited in their expression) are mutually exclusive phenomena. However, there have been strong arguments in favour of an interaction between IaSC and IeSC. Pennell & Morrow (2013) argued that IaSC and IeSC could interact in several ways, primarily as a consequence of traits involved in IeSC not being entirely sex-limited in their effects. Traits involved in IeSC could be genetically correlated with traits involved in IaSC. Alternatively, loci involved in IeSC could have pleiotropic effects with fitness consequences in the other sex (Pennell et al. 2016). Pennell and Morrow (2013) also pointed out that processes that resolve IaSC leading to evolution of sexual dimorphism, could trigger IeSC as a result of trait exaggeration. Another useful way of looking at the interaction between IeSC and IaSC is to investigate whether selection gradients on shared traits that mediate IaSC are a function of the intensity of IeSC. If the divergence in sex-specific fitness optima for shared traits is primarily driven by sexual selection (Lande 1980), experimentally increasing the intensity of IeSC (and by corollary sexual selection) should cause male and female fitness optima to move further apart, yielding a stronger signal of IaSC in that generation. However, it is important to note that, in general, there is no unequivocal theoretical expectation that strengthening IeSC should lead to a strengthening of IaSC in the population. The nature of the interaction between IeSC and IaSC will depend on the genetic architecture of traits involved in the two kinds of sexual conflict. Very few empirical studies have investigated the interaction between IaSC and IeSC. Working on *Callosobruchus maculates* isofemale lines, Berger et al. (2016) were able to show that multivariate traits associated with high male fitness were genetically associated with a greater drop in line-productivities than could be explained by mate harm (an important aspect of IeSC) or IaSC independently, pointing towards concurrent operation of IaSC and IeSC. However, to the best of our knowledge, no study has yet investigated the consequences of *experimentally* manipulating the intensity of IeSC on the signal of IaSC in the population.

In the present thesis, I explore the interaction between IeSC and IaSC in a laboratory adapted population of *Drosophila melanogaster* called LH, which is a particularly well-suited model system to investigate sexual conflict (see **Chapter 2**). I use an experimental technique called hemiclonal analysis (Abbott and Morrow 2011) first developed in *D. melanogaster* by Rice (1996). Hemiclonal analysis allows the experimenter to sample a panel of entire haploid genomes (or "hemigenomes"), with the exception of chromosome

IV (the dot chromosome), from a source population. These hemigenomes can then be expressed in males and females carrying the rest of the genome randomly sampled from the same source population to create male and female "hemiclones". This allows explicit measurements of additive genetic (co)variances for various traits. Using hemiclonal analysis, I sample a panel of 39 hemigenomes from the LH populations (**Chapter 2**).

In **Chapter 3**, I specifically address the following question: Does experimentally changing the intensity of IeSC affect the intensity of IaSC in the population? I measure the contribution of each of the 39 hemigenome lines to male and female adult reproductive fitness at three adult sex ratios: 3:1 female biased sex ratio (weak IeSC), 1:1 equal sex ratio (intermediate IeSC) and 1:3 male biased sex ratio (strong IeSC). I use competitive fertilization success as the measure of male fitness and fecundity post competition for limiting amounts of supplementary live yeast as the measure of female fitness. At each sex ratio, I measure the intersexual genetic correlation for fitness and the proportion of sexually antagonistic fitness variation as proxies of the intensity of IaSC. My results suggest a statistically non-significant reduction in the intensity of IaSC at male biased and equal sex ratios compared to female biased sex ratio.

I spend a significant portion of the rest of this thesis investigating the mechanism underlying this trend. **Chapter 4** is focused on ostensibly sex-limited traits and the nature of selection acting on them at male biased, equal and female biased sex ratio. Typically, male biased sex ratios are associated with stronger sexual selection and IeSC (Gay et al. 2011; Janicke and Morrow 2018; Ł. Michalczyk et al. 2011; Nandy et al. 2013a; Nandy et al. 2013b; Wigby and Chapman 2004). However, there is a substantial body of theoretical work that suggests that male biased sex ratios may not necessarily correspond to stronger intensities of IeSC (Kokko et al. 2012; Klug et al. 2010). Therefore, I first validate whether the male biased sex ratio treatment corresponds to an increased intensity of sexual selection and IeSC relative to the female biased sex ratio treatment by measuring the sex-specific additive genetic variation for adult reproductive fitness, fecundity of females and the selection gradients on male reproduction related traits at each sex ratio. Next, I investigate whether the interaction between IaSC and IeSC is driven by genetic correlations between resistance and persistence traits. I measure several proxies of resistance and persistence in the panel of hemigenomes sampled in Chapter 2, and calculate the intersexual genetic correlations between resistance and persistence, as well as the genetic correlations between

resistance/persistence and fitness of the opposite sex. Additionally, I also investigate whether there are genetic trade-offs between various male pre- and post-copulatory traits.

In **Chapters 5a**, I investigate a set of sexually dimorphic traits that are shared between males and females; namely, locomotory activity, development time, and dry body weight. For traits that are shared between males and females, strong intersexual genetic correlations can prevent males and females from attaining their sex-specific fitness optima, particularly in presence of sexually antagonistic selection, or even strong sexual selection in one of the sexes (Lande 1980). I explore whether there is evidence of sexually antagonistic selection on any of these traits, and whether the degree of sexual antagonism depends on the sex ratio treatment. With strong sexual dimorphism and evidence of sexual antagonism in *D. melanogaster* (Long and Rice 2007; Lund-Hansen et al. 2020; Prasad et al. 2007), locomotory activity, development time, and dry body weight are a good system to address this question. First, I examine the sex-specific genetic architecture of these traits using the hemigenomes sampled in Chapter 2. I particularly ask if there is a statistically significant intersexual genetic correlation for these traits. Next, using the fitness data obtained in Chapter 3, I investigate the nature of sex-specific selection acting on these traits at female biased, equal and male biased sex ratios. In **Chapter 5b**, I extend this analysis to a multivariate trait: wing shape. *D. melanogaster* wing shape is associated with mating success in males (Menezes et al. 2013), has a strongly positive intersexual genetic correlation (Sztepanacz and Houle 2019) and has been shown to be associated with sexually antagonistic fitness variation (Abbott et al. 2010). Using 11 landmarks on the wing surface I perform geometric morphometric analyses on wings obtained from males and females expressing the hemigenomes sampled in Chapter 2. I then investigate the sex-specific genetic architecture for wing shape and then ask whether there are sex ratio-dependent signals of sexually antagonistic selection on wing shape.

Finally, in **Chapter 6**, I investigate a two-locus population genetic model for the resolution of IaSC via the evolution of modifiers that bring about sex-biased gene expression. I extend a variant of the model developed by Connallon and Clark (2010) by allowing modifier alleles to modulate the expression of both beneficial as well deleterious alleles in one of the sexes. I then quantify the efficacy of various kinds of modifier alleles at the resolution of IaSC.

To summarise, using a combination of experimentation and mathematical modelling, this thesis investigates how various kinds of intersexual genetic correlations are associated with patterns of sexual conflict. I explore the interaction between IeSC and IaSC by looking at how intersexual genetic correlations for fitness vary at different intensities of IeSC, how genetic correlations between resistance or persistence traits and traits in the opposite sex affect signals of IaSC in the population, and how intersexual genetic correlations for traits shared between males and females affect signals of IaSC at various sex ratios. Finally, with the help of a mathematical model, my thesis also addresses how intersexual genetic correlations are resolved via sex-specific gene expression.

Chapter 2

Experimental System[2]

I used the vinegar fly *Drosophila melanogaster* for the experiments outlined in the following chapters. Thomas Hunt Morgan, with the help of high-flying undergraduate students including Hermann J. Muller and Alfred Sturtevant, employed *D. melanogaster* as the model organism to establish, essentially, the bedrock of modern genetics (Schwartz 2010). In the 100 years since the publication of their landmark book, *The Mechanism of Mendelian Heredity*, *D. melanogaster* has gone from strength to strength as the model organism of choice in research areas as diverse as evolutionary biology (Flatt 2020), neurobiology (Cognigni et al. 2018), the study of insect flight (Fry et al. 2005), developmental biology (Avilés-Pagán and Orr-Weaver 2018), cancer biology (Brumby and Richardson 2005), stem cell research (Dey et al. 2016), immunology (Belmonte et al. 2020) and even space exploration (Zhang et al. 2021), to name a few. Particularly relevant to this thesis, is the role played by *D. melanogaster* in investigations of male – female evolutionary conflict. It has been at the forefront of sexual conflict research, primarily because of the tractability of long-term experimental evolution studies using *D. melanogaster,* and the development of crucial genetic tools. One such tool, hemiclonal analysis, which was first developed by Rice (1996), enables the experimenter to sample hemigenomes from the population of interest and express them in males and females carrying random genetic backgrounds from the population (Abbott and Morrow 2011). This allows explicit measurements of various quantitative genetic parameters such as additive genetic variances and covariances between quantitative traits, including Darwinian fitness. Using experimental evolution and special genetic constructs used in hemiclonal analysis (for example, "clone generator" flies; see below), *D. melanogaster* has been widely used as a model organism to investigate the evolutionary consequences of IeSC on males and females

[2] Note that portions of this chapter have been published as part of a research article (Geeta Arun, M., Chechi, T.S., Meena, R., Bhosle, S.D. and Prasad, N.G., 2022. Investigating the interaction between inter-locus and intra-locus sexual conflict using hemiclonal analysis in *Drosophila melanogaster*. BMC Ecology and Evolution, 22, Article number: 38).

(Nandy et al. 2013), quantify genetic variation for IeSC-related traits (Filice and Long 2016; Linder and Rice 2005), estimate the intensity of IaSC (Chippindale and Rice 2001; Collet et al. 2016; Ruzicka et al. 2019), identify traits involved in IaSC (Long and Rice 2007) and explore sexually antagonistic fitness consequences of male-limited or female-limited evolution (Abbott et al. 2020; Lund-Hansen et al. 2020; Prasad et al. 2007).

It is important to note that the experiments described in this thesis investigate the properties of standing genetic variation in a laboratory population of *D. melanogaster* using hemiclonal analysis. This thesis does not track the evolution of laboratory populations across time. Rather, using properties of sex-specific genetic architecture, and properties of sex-specific natural/sexual section, this thesis attempts to inform us about the potential evolutionary trajectories populations of sexually reproducing organisms can take over short evolutionary timescales.

Often evolutionary responses are seen to be distinct from phenotypically plastic responses. Yet, phenotypic plasticity, defined as the ability of an organism exhibit different phenotypes in response to different environments (Sommer 2020), can have a genetic component to it, and can itself evolve (Maggu et al. 2021). In this thesis I measure how phenotypes (primarily fitness related traits) produced by various genotypes sampled from a laboratory population of *D. melanogaster* vary in different sex ratio environments. While it is not the principal aim of this thesis, it does highlight the interrelatedness of phenotypic plasticity and evolutionary responses.

Fly populations

LH: LH is a large laboratory adapted population of *D. melanogaster*. It is a direct descendent of the population used to measure the intersexual genetic correlation for fitness ($r_{w,g,mf}$) by Chippindale and Rice (2001), and is related to the populations used by other similar studies (Collet et al. 2016; Ruzicka et al. 2019). The LH population was founded in 1991 by Dr. Larry Harshman using 400 wild inseminated females captured from an orchard near Modesto in California, US (Rice et al. 2005). The LH population is maintained on a 14-day, discrete generation cycle on a standard cornmeal-molasses diet at 25^{o}C, 50% relative humidity, and a 12-hour: 12-hour light-dark cycle. The population consists of a total of 60 vials each containing about 150 eggs in 8-10 ml food. On the 12th day post egg collection, by which time all individuals develop into adult flies, the population is randomly divided into 6 groups of 10 vials each (90 mm length × 2.5 mm diameter). Flies from each

group are mixed together in a flask and subsequently, using light CO_2 anesthesia, are sorted into 10 food-vials, each containing 16 males and 16 females. Thus, the total population size is 960 females and 960 males distributed over 60 vials. Males and females are then allowed to interact for two days in presence of limiting amounts of live yeast. On the 14^{th} day post egg-collection, both males and females are transferred to fresh food-vials (90 mm length × 2.5 mm diameter), where they are allowed to lay eggs for 18 hours. The adult flies are then discarded and the eggs are trimmed to a density of 150 per vial. These eggs then start the next generation.

In this thesis, I used the LH population to sample a panel of 39 hemigenomes (see below).

LHst: LHst was established by introgressing an autosomal, recessive and benign scarlet eye-colour marker in the LH population. Its maintenance protocol is similar to that of LH, except that the population size is half the population size of LH. LHst is regularly back-crossed to LH to replenish any genetic variation lost due to drift.

DxLH: The DxLH population was created by back-crossing the DxIV population (provided by Prof. Adam Chippindale) to the LH population for ten generations. DxLH males have a normal X chromosome and a normal Y chromosome. DxLH females have a normal Y chromosome and a compound X chromosome [C(1)DX yf]. This ensures that sons inherit their X chromosome from their father and their Y-chromosome from their mother. Both DxLH males and females have autosomes derived from LH.

Clone-generators (CG): CG males and females have a translocation between the two major autosomes (i.e. chromosome II and chromosome III) [T(2;3) rdgCst in ripPbwD] (W. R. Rice 1996a). CG females have a compound X chromosome [C(1)DX yf] and a Y chromosome. Males have a Y chromosome and an X [snsu(b)] chromosome. CG females enabled us to sample entire haploid genomes (barring the "dot" chromosome IV) and maintain them indefinitely without being damaged by recombination.

Sampling and maintaining hemigenomes

I followed a protocol of sampling and maintaining hemigenomes that was similar to the one described by (Abbott and Morrow 2011) (see Figure 2.1 for an outline). I chose forty-three males from the LH population randomly. I housed them in separate food-vials with 3 CG females each. From each of the forty-three crosses, I selected one brown-eyed male offspring. Each of these brown-eyed male offspring had a unique haploid "hemigenome"

from LH. I then crossed them with 3 CG females each. Achiasmy in male *D. melanogaster* (Satomura et al. 2019), i.e. the absence of crossovers between chromosomes during meiosis, and the unique features of CG females ensure that the sampled hemigenome gets passed on faithfully from sire to son without being recombined (with the exception of the "dot chromosome"). Each of these 43 crosses represents a unique hemigenome line. I maintained each hemigenome line subsequently by crossing 10 brown-eyed males with 20 CG females every generation. The brown-dominant and scarlet-recessive eye-colour markers on the translocation of the CG females enabled us to distinguish between males that carried the sampled hemigenomes (which were brown-eyed as they were heterozygous for the translocation) and males that were homozygous for the translocation (which were white-eyed) (Figure 2.1). See Box 2 of Abbott and Morrow (2011) for a detailed schematic. Achiasmy in males ensures that there are no recombination events between homologous chromosomes in males heterozygous for the target hemigenomes and the translocation. Recombination can happen in these males via independent assortment of chromosomes. However, "recombinant" offspring, i.e., offspring that inherit one normal chromosome II or chromosome III and a translocated chromosome from their father are not viable, as they possess an extra portion of chromosome II and a missing portion of chromosome III, or the other way around (Figure 2.1). This effectively ensures the suppression of recombination, enabling the maintenance of target hemigenomes indefinitely, with the exception of chromosome IV (the dot chromosome). Four hemigenome lines were lost in an accident. Therefore, in this thesis, I present data from 39 lines only.

Expressing target hemigenomes in males and females for experiments

Generating experimental females: In order to express hemigenomes from each line in females containing a random background from the LH population, I crossed brown-eyed males (heterozygous, carrying the target hemigenome and the translocation) with virgin LH females (Figure 2.2A). To that end, first I collected 30 vials containing 150 eggs each from the LH population. I collected the females emerging from these vials as virgins (within 6 hours of their eclosion) with the help of mild CO_2 anesthesia by sorting them into vials containing 10 females each. I combined these females with brown-eyed males from each hemigenome line. For every hemigenome line I set up three to four vials, each containing 5 males from that line and 10 virgin LH females. I allowed these males and females to interact for two days in presence of ad-libitum live yeast (to boost fecundity) and then transferred them to fresh food vials for oviposition for around 18 hours. After discarding

the adults, I trimmed the egg-density in each vial to around 250, so that the expected number of larvae surviving in each vial would be around 125. Half the eggs were expected to be unviable. This was a consequence of the fact that the males used for this cross were heterozygous for the translocation between chromosome 2 and chromosome 3. This meant that the progeny that inherited a translocated autosome along with a normal chromosome 2 or chromosome 3 from their father (expected to be 50% of the total progeny) were unviable, as they either carried an extra portion of chromosome 3, while missing a portion of chromosome 2, or the other way around (Figure 2a). I kept the expected larval density lower than the normal density in the LH population (around 150 per vial) in order to avoid overcrowding in vials that had higher than expected levels of survivorships. Red-eyed females emerging from these vials would be females carrying the target hemigenomes expressed in a random LH background. I refer to these as "focal females". Brown eyed females (which were heterozygous for the translocation) were discarded.

Generating experimental males: The protocol for generating hemiclonal males was similar, except that instead of crossing brown-eyed males from each hemigenome line to LH females, I crossed them to virgin DxLH females (Figure 2.2B). This ensured that the red-eyed male progeny emerging from these crosses (the "focal males") had the target hemigenomes expressed in a random background from the LH population. The eggs laid in the crosses between brown-eyed males from each line and DxLH females were trimmed to a density of around 500 so as to ensure the larval density would be around 125. Note that among all the zygotes from the crosses described above, half the zygotes were expected to be unviable as they either carried two Y chromosomes, or had an X chromosome in addition to a compound X chromosome. Among the remaining zygotes, half were expected to be unviable as they either carried an extra portion of chromosome III, while missing a portion of chromosome II, or the other way around, which was a consequence of the sires being heterozygous for the translocation between chromosome II and chromosome III (Figure 2b). Therefore, among all the eggs laid, only about a quarter were expected to survive.

In subsequent chapters, I describe, how focal hemiclonal flies generated as described above were used to investigate various aspects of the interaction between IaSC and IeSC.

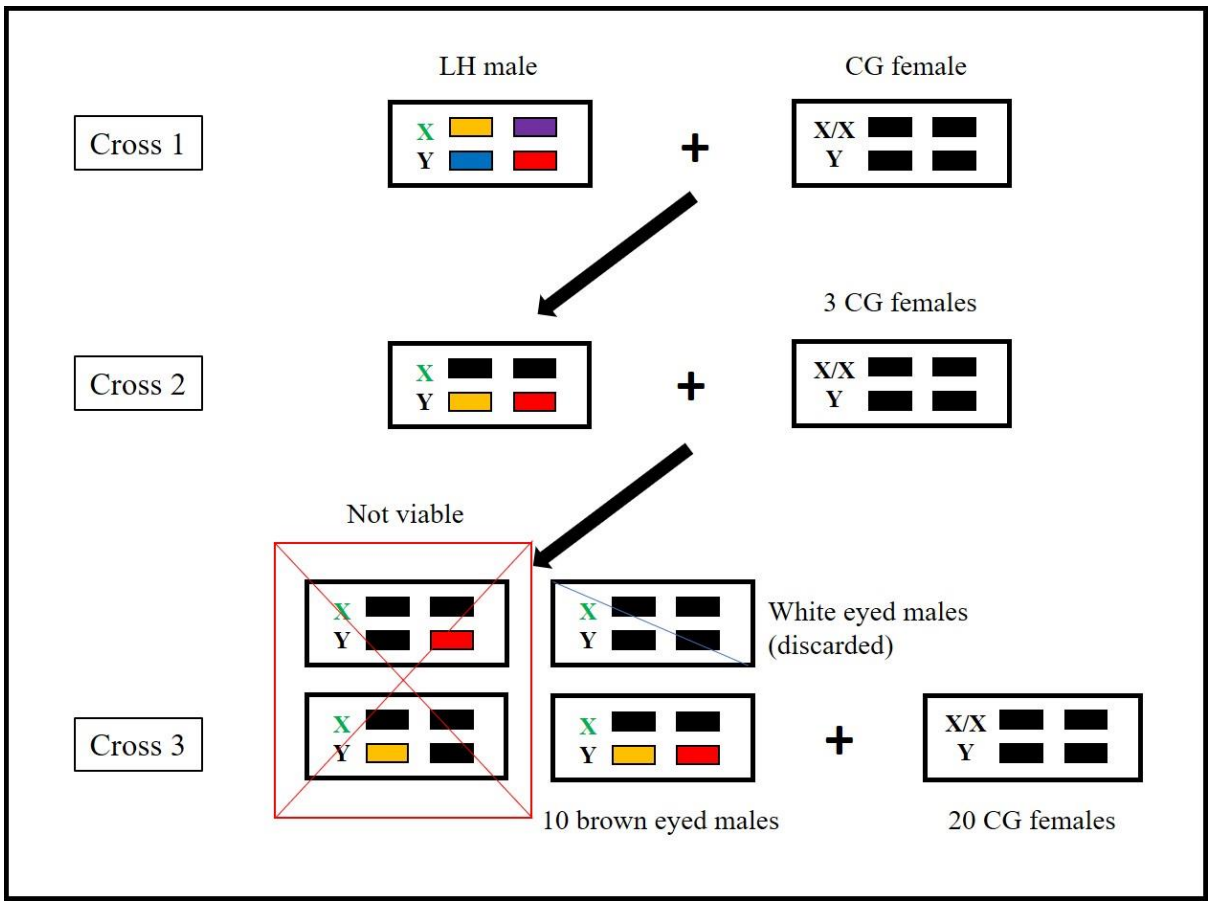

Figure 2.1. The outline of the crossing scheme used for sampling hemigenomes (adapted from Abbott and Morrow (2011)). The translocated chromosomes of the Clone Generator (CG) females are indicated by black rectangles. X/X indicates a compound X chromosome. Cross 3 was repeated every generation for maintaining the sampled hemigenomes.

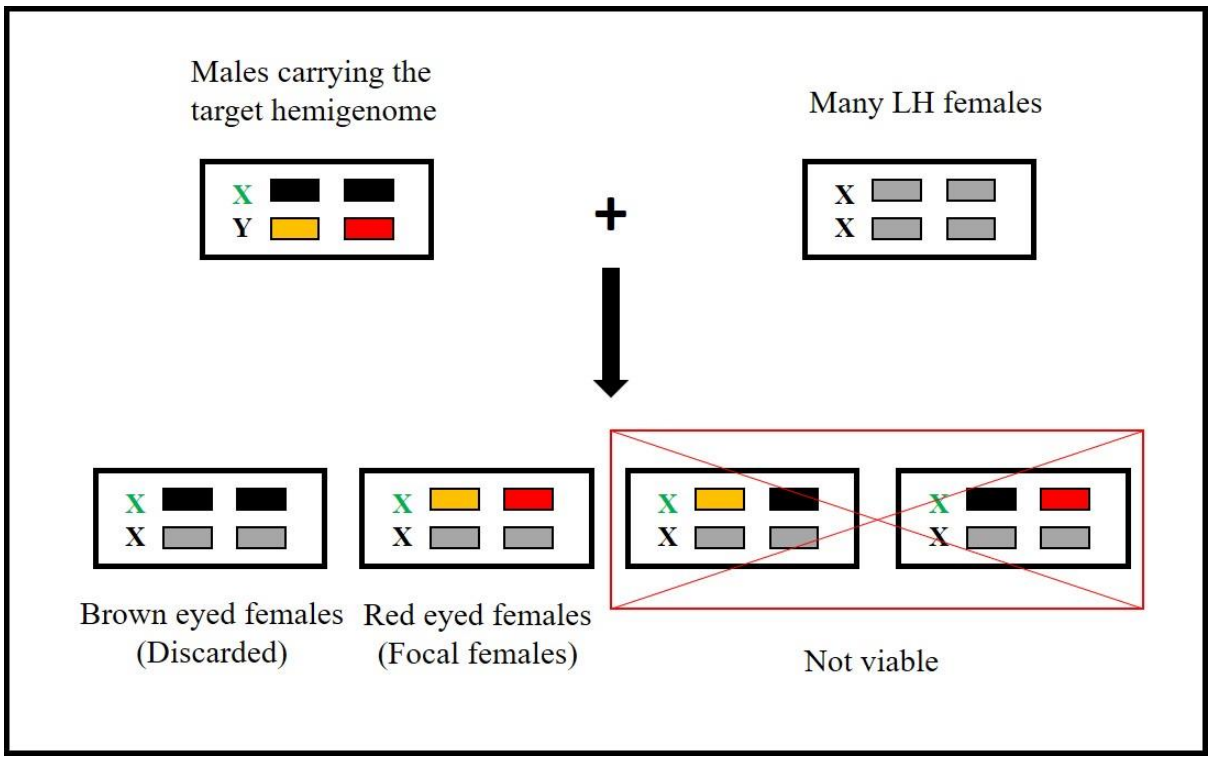

Figure 2.2A. Generating focal females carrying target hemigenomes expressed in a background randomly sampled from the LH population (adapted from Abbott and Morrow (2011)). The translocated chromosomes of the Clone Generator (CG) females are indicated by black rectangles.

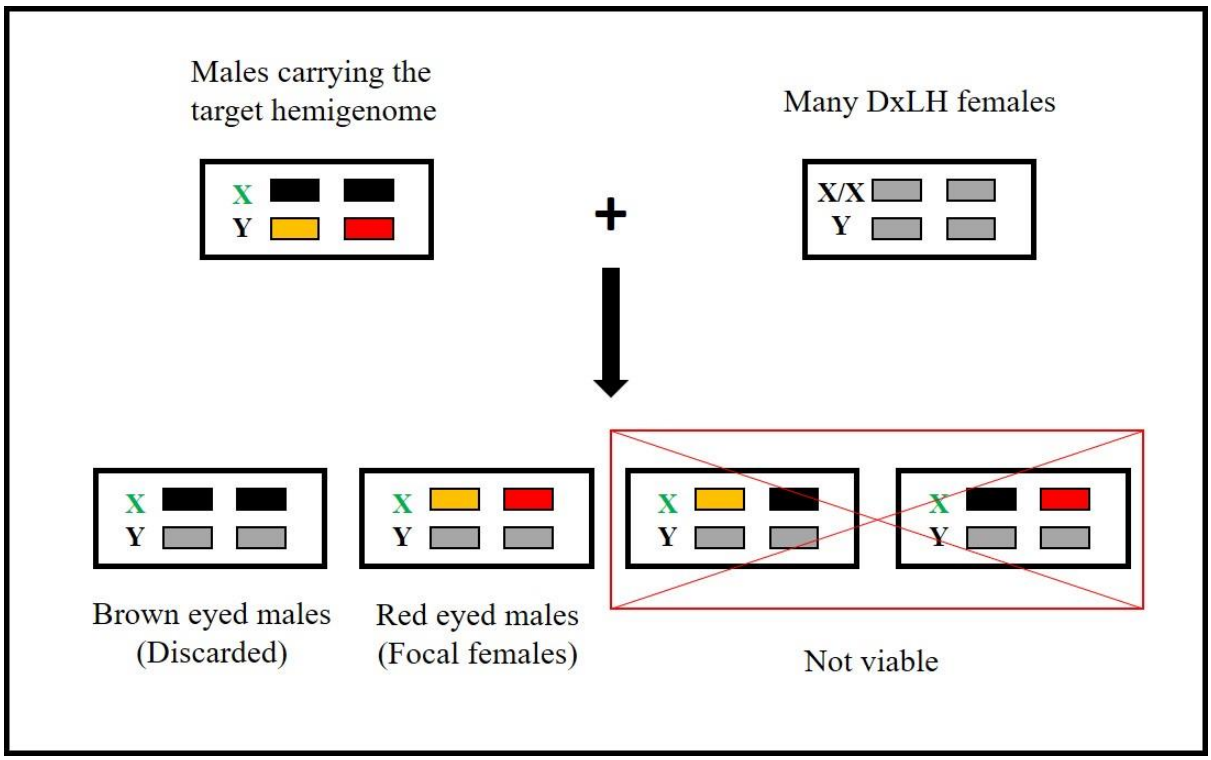

Figure 2.2B. Generating focal females carrying target hemigenomes expressed in a background randomly sampled from the LH population (adapted from Abbott and Morrow (2011)). The translocated chromosomes of the Clone Generator (CG) females are indicated by black rectangles. X/X indicates a compound X chromosome.

Chapter 3

Measuring signals of Intralocus Sexual Conflict at different intensities of Interlocus Sexual Conflict[3]

INTRODUCTION

Intralocus Sexual Conflict (IaSC) ensues when there are distinct fitness optima in males and females for traits that are expressed in both sexes and have positive intersexual genetic correlations (Bonduriansky and Chenoweth 2009; Cox and Calsbeek 2009). At the level of a locus, IaSC translates to a situation where different alleles are favoured in male and females (Cox and Calsbeek 2009). Over the last 70 years or so, numerous theoretical studies have investigated the consequences of such sex-specific, and particularly sexually antagonistic (SA), selection from the point of view of the maintenance of genetic variation (Connallon and Clark 2012; Fry 2010; Haldane 1962; Kidwell et al. 1977; Owen 1953; Parsons 1961; Rice 1996;), or from the point of view of the evolution of sexual dimorphism (Lande 1980; Matthews et al. 2019).

While the mathematical theory underlying IaSC has existed since the 1950s, empirical investigations have gathered steam only in the last two decades. Recently, with genome sequencing becoming widely accessible, a number of studies have used genomic data to investigate signatures of IaSC, without actually attempting to measure sex-specific fitness effects at the organismal level (Kasimatis et al. 2017; Mank 2017; Rowe et al. 2018). These studies have either used increased genetic diversity and/or allele frequency differentiation between the sexes to infer SA selection (Dutoit et al. 2018; Eyer et al. 2019; Lucotte et al. 2016; Stulp et al. 2012; Wright et al. 2018). However, there are a number of problems with this approach. First, SA selection is not the only evolutionary force that has the potential to result in an increased genetic diversity. Ruzicka et al. (2020) pointed out that intersexual

[3] Note that portions of this chapter have been published as part of a research article (Geeta Arun, M., Chechi, T.S., Meena, R., Bhosle, S.D. and Prasad, N.G., 2022. Investigating the interaction between inter-locus and intra-locus sexual conflict using hemiclonal analysis in *Drosophila melanogaster*. BMC Ecology and Evolution, 22, Article number: 38).

allele frequency differences can arise through such processes as sex-specific population structure, sex-specific migration and even methodological issues like incorrect assignment of sex-linked sequences to autosomes in organisms lacking high quality reference genomes (Bissegger et al. 2020). Furthermore, theoretical work suggests that effects of selection can readily be masked by noise due to sampling error for modest sample sizes (Kasimatis et al. 2017; Ruzicka et al. 2020), unless selection coefficients are astonishingly large (Eyer et al. 2019). Second, SA selection can generate allele frequency differences between males and females only in the case of viability selection. A meta-analysis of studies investigating sex-specific selection suggests that SA selection is, more often than not, a consequence of selection operating at the reproductive stage (Cox and Calsbeek 2009). Studies seeking to detect allele frequency differences between the sexes as a signal of IaSC will not be able to detect IaSC arising due to selection other than viability selection. Therefore, while genomic approaches have the potential to uncover signals of SA selection in systems where directly measuring fitness is difficult, they are unlikely to replace direct measurements of fitness and traits at the organismal level.

Empirical studies of IaSC at the organismal level have, typically, taken one of three different approaches. First, a large number of studies directly measured sex-specific selection gradients or selection differentials (reviewed in Cox and Calsbeek (2009)). These studies detected SA selection acting on several different kinds of traits including life-history (Berger et al. 2016; Holman and Jacomb 2017; Lewis et al. 2011), morphological (Berger et al. 2016; Delph et al. 2011), behavioural (Long and Rice 2007), and even immunocompetence related (Svensson et al. 2009) traits. Second, some studies employed experimental evolution in *Drosophila melanogaster* to restrict the operation of selection to only one of the sexes and asked whether this leads to an increase in the fitness of the selected sex, accompanied by a decrease in the fitness of the unselected sex (Abbott et al. 2020; Lund-Hansen et al. 2020; Morrow et al. 2008; Prasad et al. 2007). A third set of studies investigated sex-specific genetic architecture for measures of Darwinian fitness in wild or laboratory populations. These studies primarily used the intersexual additive genetic correlation for fitness ($r_{gw,mf}$) as the signal of IaSC, with low or negative values of $r_{gw,mf}$ assumed to be indicative of strong IaSC. Some such studies used parent-offspring correlations to estimate $r_{mf,gw}$ with most detecting strongly negative estimates of $r_{gw,mf}$ (Fedorka and Mousseau 2004; Foerster et al. 2007; Pischedda and Chippindale 2006) (but see Pischedda and Chippindale (2017)). Others employed hemiclonal analysis or within-

generation quantitative genetic breeding designs to estimate $r_{gw,mf}$ with estimates of $r_{gw,mf}$ varying from significantly negative (Chippindale and Rice 2001; Collet et al. 2016; Innocenti and Morrow 2010), to indistinguishable from 0 (Berger et al. 2014; Collet et al. 2016; Ruzicka et al. 2020), to significantly positive (Berger et al. 2014). While $r_{gw,mf}$ is a popular metric of the strength of IaSC, it is not without its limitations. Using a quantitative genetic model, Connallon and Matthews (2019) showed that SA selection is a necessary but not a sufficient condition for negative values of $r_{gw,mf}$. They showed that negative values of $r_{gw,mf}$ arise when the magnitude of SA selection is strong, relative to the sex-specific additive genetic variances for the trait under SA selection. Some recent studies have used a slightly different metric for the strength of IaSC: the proportion of sexually antagonistic fitness variation (Berger et al. 2014). This involves measuring male and female fitnesses for target genotypes (i.e., isofemale lines or hemiclones). The coordinate system made up of male and female fitness scores is then rotated 45^{o} such that the new y-axis is the axis of SA fitness variation, while the new x-axis is the axis of sexually concordant fitness variation (see Figure 3.1 for a schematic). The proportion of fitness variation distributed along the SA axis gives a direct metric of IaSC in the population.

While a staggering number of empirical studies have attempted to measure signals of IaSC, only a handful of studies have compared signals of IaSC in different environments (Berger et al. 2014; Delcourt et al. 2009; Holman and Jacomb 2017; Punzalan et al. 2014), or between different replicates of the same population evolving independently (Collet et al. 2016). This is an important issue, because theoretical work suggests that signals of IaSC are not static, but can respond plastically to environmental change, and even evolve over longer time scales. There are two competing phenomena that can affect the direction of the evolution of signals of IaSC. First, SA selection can, in some cases, drive the evolution of sex-specific genetic architecture through a variety of different mechanisms (Connallon and Clark 2010; 2011; Day and Bonduriansky 2004; Spencer and Priest 2016) weakening the signals of IaSC in the population. Alternatively, as populations adapt to a novel environment, as long as there are intersexual genetic correlations in mutational effects, the angle between selection vectors in males and females, and therefore the strength of IaSC in the population is expected to increase (Connallon and Clark 2014). A testable prediction of this theory is that if a well-adapted population is exposed (for a single generation) to a novel environment, the signals of SA selection should be inflated in the native environment, relative to the novel environment (Connallon and Hall 2018). This idea has been tested in

insects by numerous studies, with some studies finding evidence in support of the idea (Berger et al. 2014; Long et al. 2012), while others either failed to detect any effect of change of environment on the degree of sexual antagonism (Holman and Jacomb 2017; Martinossi-Allibert et al. 2018) or reported an increase in sexual antagonism in novel environments (Delcourt et al. 2009; Punzalan et al. 2014). Most of these studies have focused on measuring changes in the signals of IaSC upon altering the food source or subjecting the population to an environmental stress. An important aspect of the environment is the mating system of the population itself, particularly the strength of sexual selection and Interlocus Sexual Conflict (IeSC) to which the population is exposed. IeSC is typically thought to be a conflict over the outcomes of reproductive interactions between males and females, and is modelled over traits that are sex-limited in their effects: "resistance" traits in females, and "persistence" traits in males (Rowe et al. 2005) (see Chapter 1). While in their preliminary mathematical formalisms, IaSC and IeSC are mutually exclusive, there are several ways in which the two can interact (see Chapter 1). First, resistance and persistence traits could be genetically correlated, or have pleiotropic fitness effects when expressed in the opposite sex (Pennell and Morrow 2013). Second, given that most evidence of SA selection involves selection operating on reproduction related traits (Cox and Calsbeek 2009), any change in the strength of sexual selection and/or IeSC is likely to affect the signals of IaSC in the population. Unfortunately, very few studies have explicitly sought to measure signals of IaSC upon experimentally changing in the intensity of IeSC in the population.

In this chapter, I attempt to address this gap in our knowledge by using the 39 hemigenomes sampled from the LH population in Chapter 2. I measured the reproductive fitness of males and females carrying each hemigenome (expressed in a large number of genetic backgrounds randomly sampled from the LH population) at three different adult sex-ratios: male-biased (strong IeSC), equal (intermediate IeSC) and female-biased (weak IeSC). Manipulating operational sex-ratios has been one of the two principal techniques of experimentally changing the intensity of IeSC (Michalczyk et al. 2011; Nandy et al. 2013a; 2014; Wigby and Chapman 2004; also see Janicke and Morrow (2018)), the other being experimentally enforcing monogamy (Crudgington et al. 2010; Demont et al. 2014; Gay et al. 2011; Holland and Rice 1999; Hosken et al. 2001; Tilszer et al. 2006). First, I examined the relationship between the contribution of each hemigenome to sex-specific fitness at each of the three adult sex ratios. Particularly, I attempted to infer if there were any

interactions between hemigenome line, sex and sex ratio for fitness. Subsequently, I estimated the following two parameters corresponding to the strength of IaSC for each sex-ratio: the intersexual additive genetic correlation for fitness ($r_{gw,mf}$), and the proportion of sexually antagonistic fitness variation. If increasing the strength of IeSC leads to a strengthening of IaSC, one would expect $r_{gw,mf}$ to be lower, but the proportion of sexually antagonistic fitness variation to be higher at the male biased sex ratio.

METHODS

Using cytogenetic cloning techniques (Rice 1996), I sampled a panel of 39 hemigenomes from a laboratory adapted population of *D. melanogaster* called LH (see Chapter 2). This involved using Clone Generator (CG) females that possess a compound X chromosome and a translocation between the two major autosomes. This allows the sampling and cloning of entire haploid nuclear genomes (with the exception of the dot chromosome). I expressed each of these haploid genomes in males and females carrying the rest of the genome randomly sampled from the LH population. Subsequently, I measured male and female fitness at three different intensities of IeSC obtained by varying the adult sex ratio: male biased sex ratio (24 males: 8 females per vial) where IeSC is expected to be intense, female biased sex ratio (8 males: 24 females per vial) where IeSC is expected to be weak, and equal sex ratio (16 males: 16 females per vial) where the intensity of IeSC is expected to be intermediate. I used competitive fertilization success as the measure of male fitness, and fecundity post strong female – female competition for acquiring live yeast as the measure of female fitness.

Female fitness assay

I followed the protocol described in Chapter 2 for generating focal females for this experiment. Briefly, I crossed brown eyed males from different hemigenome lines, that were heterozygous for target hemigenomes and the translocation, with virgin LH females. I trimmed the eggs laid by LH females to around 250 per vial, so as to ensure that the larval density was around 125 per vial (see Chapter 2 for an explainer). On the day the eggs from the crosses were trimmed, I also collected 100 vials of 150 eggs each from the LHst population. I collected focal females (red-eyed female progeny emerging from the crosses described above) as virgins using light CO_2 anesthesia and held them in food-vials at a density of 8 females per vial. On the 12^{th} day post egg collection, I set up adult competition food-vials (90 mm length × 2.5 mm diameter) supplemented by 100 μL of live- yeast

suspension in water. The concentration of the yeast suspension was adjusted according to the sex-ratio treatment such that the per-female yeast availability in the vial was always 0.47 mg. In these adult competition vials, I combined the focal females with competitor LHst females and LHst males in appropriate numbers depending on the sex-ratio treatment. Regardless of the sex-ratio treatment, the total number of flies (males + females) in a vial was always 32, and the ratio of focal females to competitor females was always 1:3. For the male-biased sex-ratio, each vial had 24 LHst males, 2 focal females and 6 LHst competitor females. The equal sex ratio had 16 LHst males, 4 focal females and 12 LHst competitor females in each vial. The female biased sex-ratio had 8 LHst males, 6 focal females and 18 LHst competitor females. I allowed males and females to interact in the adult competition vials for two days. Subsequently, from each vial (regardless of the sex-ratio) I transferred two focal females to a fresh food-vial for egg-laying. I discarded these females after 18 hours and counted the eggs laid in that period, which was used as a measure of the fitness of the focal females in that vial. I performed two replicate assays for each of the sex-ratios, all on separate days. For each replicate assay of each sex-ratio I set up 7 adult competition vials for every hemigenome family. However, due to experimental contingencies, in some cases I had to set up fewer than 7 adult competition vials for some hemigenome lines. Overall, I assayed the fecundity of nearly 3276 females (39 lines × 3 sex ratios × 2 replicate assays × 7 adult competition vials × 2 females from each adult competition vial).

Male fitness assay

In order to generate focal males for this experiment, I crossed brown eyed males from each hemigenome line to virgin DxLH females. I trimmed the eggs laid by these females to around 500 per vial, so that the expected larval density was 125 per vial (see Chapter 2 to for an explanation). Additionally, on the day the eggs from these crosses were trimmed, I also collected 100 vials of 150 eggs each from the LHst population to generate competitor males and females for the fitness assay. I collected focal males (red-eyed male progeny emerging from the crosses described above) as virgins in food-vials in groups of 8. I also collected as virgins LHst females in groups of 8 per food-vial and competitor LHst males in groups of 6 per vial. On the 12th day post egg collection, I set up adult competition vials (90 mm length × 2.5 mm diameter) as described for the female-fitness experiment. I then combined focal males, competitor LHst males and LHst females in the adult competition vials in appropriate numbers based on the sex-ratio (Male-biased: 6 focal males, 18 LHst

competitor males, 8 LHst females; Equal: 4 focal males, 12 LHst competitor males, 16 LHst females; Female-biased: 2 focal males, 6 LHst competitor males, 24 LHst females). I let the flies interact in the adult competition vials for two days. On the 14th day post egg collection, from each vial I transferred 7 randomly chosen LHst females individually into separate test-tubes (diameter 0.5 mm, length 10 mm) containing food for oviposition. After 18 hours, I discarded the females and incubated the test tubes in standard maintenance conditions. Twelve days later, when all progeny in the test tubes had developed into adults I froze the test-tubes at -20ºC. I scored the progeny from each test-tube for their eye colour. The proportion of red-eyed progeny among all the progeny from the 7 test tubes corresponding to a vial was used as the measure of the fitness of focal males from that vial. For males too, I performed two replicate assays for each of the sex-ratio-treatments, with all six assays being set up separately. Within each assay, for every sex-ratio treatment, I set up 5 adult competition vials for every hemigenome family. In some cases, there were fewer than 5 adult competition vials. See Supplementary Information I for details. Thus, in total, I scored the progeny for eye colour from nearly 8190 females (39 lines × 3 sex ratios × 2 replicate assays × 5 adult competition vials × 7 females from each adult competition vial).

Statistical Analysis

All analyses were performed in R version 4.0.2.

In order to examine if there was a statistically significant effect of hemigenome line and its interaction with sex and sex ratio, I used the R packages "lme4" (Bates et al. 2022) and "lmerTest" (Kuznetsova et al. 2020) to fit the following linear mixed effects model on male and female fitness data scaled and centred separately for each day of the experiment:

Standardised Fitness ~ Sex + Sex.Ratio + Sex:Sex.Ratio + (1|Hemigenome line) + (1|Hemigenome line:Sex) + (1|Hemigenome line:Sex.Ratio) + (1|Hemigenome line:Sex:Sex.Ratio).

In order to calculate the $r_{gw,mf}$ I calculated the mean fitness associated with hemigenome line in both males and females. To that end first I arcsin-square-root transformed the male fitness data for each adult competition vial. I divided the data for each day by the mean fitness of that day. Since, I had performed two replicate fitness assays for each sex-ratio with multiple measurements on each day, I calculated the average fitness for hemigenome lines for each sex-ratio in two steps. For both males and females, for each sex ratio, I first calculated the average fitness for each hemigenome line on each of the two replicate days

and then calculated the average of the two averages. I then scaled and centred the data for each sex × sex-ratio combination separately. First, I used this data to calculate genetic correlations for sex-specific fitness across sex ratios. I then calculated the intersexual genetic correlation for fitness ($r_{w,g,mf}$) for each sex-ratio. Following Berger et al. (2014) and Ruzicka et al. (2019), I also calculated the proportion of fitness variation along the sexually antagonistic axis by rotating the original coordinate system represented by a female fitness axis (X-axis) and a male fitness axis (Y-axis) by 45º in the anti-clockwise direction. As a result of this transformation the new X-axis is the axis of sexually concordant fitness variation, while the new Y-axis is the axis of sexually antagonistic fitness variation. I used the following matrix operation separately for the scaled and centred data for each sex-ratio: $\begin{pmatrix} \bar{W}_{C,i} \\ \bar{W}_{A,i} \end{pmatrix} = \begin{pmatrix} 1/\sqrt{2} & 1/\sqrt{2} \\ -1/\sqrt{2} & 1/\sqrt{2} \end{pmatrix} \begin{pmatrix} \bar{W}_{F,i} \\ \bar{W}_{M,i} \end{pmatrix}$, where $\bar{W}_{C,i}$ and $\bar{W}_{A,i}$ are the sexually concordant and sexual antagonistic fitness components respectively for the hemigenome line i for that sex ratio, and $\bar{W}_{F,i}$ and $\bar{W}_{M,i}$are the average female and male fitnesses respectively for the hemigenome line i for that sex ratio. I then calculated the proportion of variance in fitness lying along the sexually antagonistic axis for each sex ratio.

In order to calculate 95% confidence intervals around our estimates of across sex ratio correlations for sex-specific fitness, $r_{w,g,mf}$ and AI I used a stratified bootstrap approach using the R package "boot" (Canty et al. 2010). For each sex-ratio, I created 10000 data-sets by sampling with replacement within each sex × hemigenome line × day combination. This procedure ensured that each of the bootstrapped data-sets had representation from each sex × hemigenome line × day combination in the same proportions as the original data-set. I also calculated 95% confidence intervals for differences between $r_{w,g,mf}$ and AI estimates of male-biased and female-biased sex ratios to test if they included 0.

Following Ruzicka et al. (2019), I used the R package "MCMCglmm" (Hadfield 2010) to fit a Bayesian linear mixed effects model using Monte Carlo sampling methods to estimate across sex ratio correlations for sex-specific fitness, $r_{w,g,mf}$ and male and female heritabilities for each sex-ratio separately. I first scaled and centred arcsin-squareroot transformed male fitness data and female fitness data separately for each day. I fit the following model for each sex-ratio: $W_{ijkmn} \sim S_i + R_j + S.R_{ij} + L_{ijk} + D.L_{km} + \varepsilon_{ijkmn}$, where W_{ijkmn} is the scaled and centered fitness of adult-competition vial n of sex i, sex ratio j, and hemigenome line k on day m. S_i, R_j and $S.R_{ij}$ represent the fixed effects of sex, sex ratio and their interaction. L_{ijk} represents a term corresponding to the sex-specific random effect

of each hemigenome line for sex ratio j. $D.L_{km}$ represents a scalar corresponding to the random interaction of day and hemigenome line. L_{ijk} is modelled to follow a multivariate normal distribution with a mean 0, and whose variance-covariance matrix is given by the additive genetic variance in female fitness ($\sigma^2{}_{w,g,f}$) and male fitness ($\sigma^2{}_{w,g,m}$) in each of the three sex ratios; the intersexual genetic covariance for fitness ($Cov_{w,g,mf}$) for each of the three sex ratios; as well as sex-specific genetic covariances for fitness between male biased and female biased sex ratio ($\sigma^2{}_{w,g,mb-fb}$), between male biased and equal sex ratio ($\sigma^2{}_{w,g,mb-e}$), and between female biased and equal sex ratio ($\sigma^2{}_{w,g,e-fb}$); along with other terms corresponding to genetic covariances for fitness across sex and sex ratios both. ε_{ijkmn} represents the sex and sex-ratio specific residuals. ε_{ijkmn} is modeled to follow a normal distribution with a mean 0 and variance given by the sex and sex-ratio specific residual fitness variance ($\sigma^2{}_{w,r,m}$ for males and $\sigma^2{}_{w,r,f}$ for females for each of the three sex-ratios). I used these estimates to calculate the following sex- or sex ratio-specific quantitative genetic parameters:

1. Genetic covariance for fitness between male biased and female biased sex ratio in sex i, $r_{w,g,mb-fb,i} = \frac{Cov_{w,g,mb-fb,i}}{\sqrt{\sigma^2{}_{w,g,fb,i}}\sqrt{\sigma^2{}_{w,g,m,i}}}$

2. Genetic covariance for fitness between male biased and equal sex ratio in sex i, $r_{w,g,mb-e,i} = \frac{Cov_{w,g,mb-e,i}}{\sqrt{\sigma^2{}_{w,g,mb,i}}\sqrt{\sigma^2{}_{w,g,e,i}}}$

3. Genetic covariance for fitness between equal and female biased sex ratio in sex i, $r_{w,g,e-fb,i} = \frac{Cov_{w,g,e-fb,i}}{\sqrt{\sigma^2{}_{w,g,e,i}}\sqrt{\sigma^2{}_{w,g,fb,i}}}$

4. Heritability for female fitness in sex ratio j, $h^2_{w,f,j} = \frac{\sigma^2{}_{w,g,f,j} \times 2}{\sigma^2{}_{w,r,f,j} + \sigma^2{}_{w,g,f,j}}$

5. Heritability for male fitness in sex ratio j, $h^2_{w,m,j} = \frac{\sigma^2{}_{w,g,m,j} \times 2}{\sigma^2{}_{w,r,m,j} + \sigma^2{}_{w,g,m,j}}$

6. Intersexual genetic correlation for fitness in sex ratio j, $r_{w,g,mf,j} = \frac{Cov_{w,g,mf,j}}{\sqrt{\sigma^2{}_{w,g,f,j}}\sqrt{\sigma^2{}_{w,g,m,j}}}$

RESULTS

Interactions between hemigenome line, sex, and sex ratio

The output of my linear mixed effects model (Table 3.1) suggested that there was a statistically significant effect of hemigenome line (likelihood ratio test (LRT), $p = 0.0237$), its interaction with sex (LRT, $p < 0.0001$), and the three-way interaction between hemigenome line, sex and sex ratio (LRT, $p = 0.0002$). While all across-sex ratio correlations for both males and females, and all across-sex correlations for all three sex ratios were positive (Table 3.2A-B, Figure 3.2, Figure 3.3). many hemigenome lines exhibited fitness rank reversals across sex ratios (Figure 3.4) or sex (Figure 3.5), explaining the interactions observed in the linear mixed effects model.

Signals of IaSC at male biased, equal, and female biased sex ratios

The analyses using hemigenome line averages suggested that the $r_{w,g,mf}$ for male biased sex-ratio, equal sex ratio, and female biased sex ratio were (0.3805, 95% CI = [0.2992, 0.52833]), (0.4027, 95% CI = [0.3140, 0.5526]) , and (0.2515, 95% CI = [0.1198, 0.4502]), respectively. While the estimate of $r_{w,g,mf}$ at male biased sex ratio was lower than at female biased sex ratio, the 95% confidence intervals (CIs) for this difference (-0.0721, 0.3507) included 0, suggesting these patterns were not statistically significant. The estimates of $r_{w,g,mf}$ from the MCMCglmm model (Table 3.2B) were slightly higher but the relative trend among sex-ratios was similar. The credible interval for the difference between the $r_{w,g,mf}$ estimates for male biased and female biased sex ratios (-0.3788, 0.5561) included 0, suggesting the two were not significantly different. The $r_{w,g,mf}$ estimates were comparable for male biased (0.5056, 95% credible intervals (CI) = [0.1418, 0.7983]) and equal sex-ratios (0.4999, 95% CI = [0.1397, 0.7787]), while the $r_{w,g,mf}$ estimate for the female biased sex ratio (0.4462, 95% CI = [0.0059, 0.8470]) was lower (Table 3.2B).

Similarly, the 95% CIs for the difference between estimates of the proportion of sexually antagonistic fitness variation for male biased and female biased sex-ratios (-0.1753, 0.0360) included 0, suggesting these differences were not statistically significant. Nevertheless, the proportion of fitness variation along the sexually antagonistic axis (estimated using line averages) was comparable for male biased and equal sex ratios (0.3097, 95% CI = [0.2358, 0.3504] and 0.2986, 95% CI = [0.2237, 0.3430] respectively), but higher at the female biased sex ratio (0.3742, 95% CI = [0.2749, 0.4401]).

Male and female heritabilities at male biased, equal, and female biased sex ratios

The estimates of female heritabilities for fitness, obtained using the MCMCglmm model, in male biased (0.8702, 95% CI = [0.5935, 1.1520]), equal (0.9992, 95% CI = [0.7337, 1.2696]) and female-biased (0.7385, 95% CI = [0.5021, 1.0539]) sex ratios, were higher than the corresponding estimates of male heritabilities at male biased (0.4788, 95% CI = [0.2383, 0.7303]), equal (0.5762, 95% CI = [0.3192, 0.8637]) and female biased (0.2229, 95% CI = [0.0495, 0.4080]) sex ratios. This trend was statistically significant, as the 95% credible intervals for the difference in female and male heritabilities did not overlap with 0 in male biased [-0.7343, -0.0207] and equal [-0.7703, -0.0852] sex ratios, but not in the female biased sex ratio [-0.3740, 0.0668]. Additionally, for both males and females, equal sex-ratio had the highest heritabilities, with the male biased sex-ratio having marginally lower heritabilities. Both male and female heritabilities were considerably lower in the female biased sex-ratio. The variance estimate for the interaction between day and hemigenome line was 0.0353 (95% CI = [0.0068, 0.0606]).

DISCUSSION

I investigated the interaction between inter- and intra-locus sexual conflict in a laboratory adapted population of *D. melanogaster*. I isolated 39 hemigenomes from the LH population and measured the contribution of each hemigenome to the adult fitness of males and females at male biased, equal and female biased sex-ratios. My analyses yielded the following major findings:

(a) At each sex-ratio the intersexual genetic correlation for fitness ($r_{w,g,mf}$) was positive. $r_{w,g,mf}$ was smaller and the proportion of fitness variation along the sexually antagonistic axis higher in the female biased sex-ratio relative to male-biased or equal sex ratios, suggesting an amelioration of IaSC at higher intensities of IeSC. However, it must be noted that these differences were not statistically significant.

(b) Genetic correlations across sex ratios for male and female fitness were strongly positive.

(c) There were statistically significant hemigenome line × sex, and hemigenome line × sex × sex ratio interactions for standardized fitness.

(d) Heritabilities for fitness were the highest in the equal sex ratio, followed by the male biased sex ratio, and were considerably lower in the female biased sex-ratio.

(e) Estimates of female heritabilities in all three sex ratios were higher than the corresponding estimates of male heritabilities.

Below, I discuss the potential implications of these findings.

The interaction between IeSC and IaSC can take many different forms, primarily as a consequence of traits involved in one kind of conflict also playing a role in the other kind of conflict (Pennell and Morrow 2013). While there is no universal expectation with respect to the direction in which these interactions should proceed, in some of the cases, IaSC and IeSC are expected to reinforce each other. For example, at higher intensities of IeSC, stronger sexual selection could result in male and female fitness optima for shared traits being further apart leading to a stronger signal of IaSC, relative to lower intensities of IeSC. As a hypothetical example imagine a sexually selected trait that is exaggerated in males as a result of sexual selection, but has costs in terms of natural selection. As a result, the male optimum for the trait is higher than for females, which are primarily subjected to natural selection. If the strength of sexual selection in the population increases (possibly, if the sex ratio becomes more male biased), the male optimum would become higher owing to increased sexual selection, while the female optimum would be unaffected. This would create a greater dissonance between male and female optima, leading to stronger IaSC. Similarly, if traits involved in IeSC have negative fitness consequences when expressed in the opposite sex (e.g., the genes that code for trait exaggeration in males in the hypothetical example above, have negative effects on fitness when expressed in females) similar to the assumptions of Pennell et al. (2016), experimentally increasing the intensity of IeSC, all else being equal, would lead to an increase in the signal of sexually antagonistic selection (relative to sexually concordant selection). My results find no evidence that the interaction between IaSC and IeSC manifests along these lines. In contrast, I find a statistically non-significant *decrease* in the signal of IaSC at higher intensities of IeSC. The proportion of sexually antagonistic variation was higher at the female biased sex ratio, compared to the other two sex ratios. While the absolute estimates of $r_{w,g,mf}$ were different between the analysis using line averages and the Bayesian analysis using MCMCglmm, the relative trend among the three sex ratios was identical. Both the analyses suggested a statistically non-significant reduction in $r_{w,g,mf}$ at the female biased sex ratio compared to the male biased or equal sex ratios, which were comparable to each other.

Both IaSC and IeSC are complex biological phenomena that involve an interplay of a large number of traits. To be able to predict how changing the intensity of one, influences the intensity of the other would, therefore, require an understanding of the genetic architecture of these traits, and nature of selection acting on each of them. Below, I describe two plausible scenarios under which strengthening the intensity of IeSC could lead to weaker IaSC within the population.

First, as the intensity of IeSC increases, it is possible that selection gradients on traits involved in IaSC change, leading to a change in the intensity of IaSC over those traits. In an extreme scenario, with increase in the strength of IeSC, one of these selection gradients could change signs in one of the sexes resulting in sexually concordant selection on that trait. Given that I found a strong three-way interaction between sex, sex ratio, and hemigenome line for fitness in my linear mixed effects model, this explanation becomes fairly plausible. Below, I use available results about locomotory activity to illustrate my point. Adult locomotory activity has been shown to mediate IaSC in *D. melanogaster* (Long and Rice 2007), with more active males and less active females enjoying higher fitness. Numerous studies have reported patterns that indicate that *D. melanogaster* males that tend to be more active enjoy greater mating success (Hall 1994; Jordan et al. 2006; Partridge et al. 1987; van Dijken and Scharloo 1979). On the other hand, female activity stimulates male courtship in *D. melanogaster* (Tompkins et al. 1982). Therefore, active females are thought to attract more courtship from males, resulting in diversion of resources away from egg-production. While a substantial fraction of fitness costs of male – female interactions to females are pre-mating (Partridge and Fowler 1990), several studies have highlighted post-mating fitness costs to females (Fowler and Partridge 1989; Parker et al. 2013; Wigby and Chapman 2005). Therefore, it is possible that in an environment where IeSC is intense (for example, the male-biased sex-ratio in my experiments), where male-courtship is guaranteed regardless of female activity, selection on females to reduce the number of matings might be stronger than avoiding courtship per se. As a corollary, in an environment with extremely elevated levels of male-courtship, more active females might enjoy higher fitnesses by virtue of their ability to reject male mounting attempts. Therefore, at higher intensities of IeSC, the selection on adult locomotory activity might become sexually concordant reducing the intensity of IaSC. Nandy (2012) and Nandy et al. (2013a) evolved replicate populations of *D. melanogaster* at male-biased, equal and female-biased sex-ratios, and reported that both males and females from the male-biased population evolved

to become more active than their counterparts evolving under equal and female-biased sex ratios. This suggests that at male-biased sex-ratio, where levels of IeSC are the highest, the IaSC over locomotory activity seems to be weakened, if not removed entirely, so as to permit the evolution of increased locomotory activity levels in both males and females.

Second, increasing the strength of IeSC could ameliorate IaSC if male and female traits (unfortunately called "persistence" and "resistance" traits respectively) involved in IeSC are positively genetically correlated. If the most "resistant" females preferentially mate with the most "persistent" males a positive linkage disequilibrium between "resistance" and "persistence" could build up in the population. As the strength of IeSC increases, by definition, the strength of selection on "persistence" and "resistance" traits increases. If the two sets of traits are positively genetically correlated, this would result in an increase in the strength of sexually concordant selection; all else being equal, this would yield a weakened IaSC signal. Rice et al. (2005) could not find a statistically significant correlation between male and female remating rates in a laboratory population of *D. melanogaster*. However, they did not explicitly observe mating, but measured mating rates in terms of the proportion of females in a vial that remated after their first mating. There are several alternative ways of measuring proxies of persistence and resistance including measuring the latency between the first and the second mating, explicit observations to record matings or measuring courtship related behaviours in males and females. It remains to be explored if these traits are genetically correlated in the panel of hemigenomes used in this thesis.

My study is also relevant in the context of the "evolutionary inevitability of sexual antagonism". Connallon and Clark (2013) used a variant of Fisher's geometric model to show that as populations adapt to their environments the degree of sexual antagonism in the populations should increase. Consequently, if a population that is well-adapted to its environment is exposed to a novel environment, the degree of sexually antagonistic selection experienced by the population should be lower (Connallon and Hall 2018). This idea has been tested in insects by numerous studies, with some studies finding evidence in support of the idea (Berger et al. 2014; Long et al. 2012), while others either failed to detect any effect of change of environment on the degree of sexual antagonism (Holman and Jacomb 2017; Martinossi-Allibert et al. 2018) or reported an increase in sexual antagonism in novel environments (Delcourt et al. 2009; Punzalan et al. 2014). In my case the LH population has been maintained in the laboratory for >500 generations at equal sex-ratio. Therefore, male-biased and female-biased sex-ratios represent novel environments to

which the population is not expected to have adapted. My results do not provide any evidence in favour of the idea that maladapted populations should exhibit weaker IaSC. I found that compared to equal sex-ratio, male biased sex ratio exhibited a comparable intensity of IaSC, while the female biased sex-ratio resulted in a statistically non-significant *increase* in the strength of IaSC (lower $r_{w,g,mf}$ and higher proportion of sexually antagonistic fitness variation). One of the reasons why I could not detect a clear increase in the strength of IaSC in my novel environments (male biased and female biased sex ratio) could be the fact that the sex ratio treatments used in this study were applied only for two days in the adult stage of the flies. This duration is fairly short, compared to the life cycle of the LH population (14 days). Therefore, it could be argued that the novel environments (male biased and female biased sex ratio) were not sufficiently novel. However, this explanation is unlikely for two reasons. First, while two days is indeed a short period compared to the entire life cycle of the LH population, the period between day 12 and day 14, when sex ratio treatments were applied in my experiments, is a crucial phase for the reproductive fitness of LH flies. Eggs laid in the 18 hours post day 14 contribute to the next generation. Additionally, there is strong last male sperm precedence in *D. melanogaster* (Schnakenberg et al. 2012). Therefore, male – female interactions from day 12 through day 14 are crucial determinants of both male and female fitness, and also, potentially, mediate IeSC in the LH population (Rice et al. 2005). The LH population has been maintained using the current protocol for more than 500 generations. Therefore, the period between day 12 and day 14 in the LH life cycle is, perhaps, the most ecologically relevant phase to perform adult-stage experimental manipulations. Second, I found a strong three-way interaction between sex, sex ratio, and hemigenome line ($p = 0.0002$) for reproductive fitness. This clearly suggests that the three sex ratio environments are different in terms of how sex-specific selection operates in them.

At each of the three sex-ratios my estimates of $r_{w,g,mf}$ were strongly positive. This is in sharp contrast to Chippindale and Rice (2001) who had reported a negative $r_{w,g,mf}$ in the ancestral population of the LH population used by us. In fact, several studies have attempted to estimate $r_{w,g,mf}$ in replicates of the original LH_M population with different outcomes. Innocenti and Morrow (2010) reported a negative $r_{w,g,mf}$. Collet et al. (2016) compared $r_{w,g,mf}$ across two replicates of the LH_M population and reported that one of the replicates had a negative $r_{w,g,mf}$ while the other had an $r_{w,g,mf}$ indistinguishable from 0. Ruzicka et al (2019) sampled 200 hemigenomes from a replicate of the LH_M population and found a

positive but non-significant $r_{w,g,mf}$. Ours is the first study to report an $r_{w,g,mf}$ significantly greater than 0. While it is tempting to interpret this as evidence indicating resolution of IaSC through the traditional pathway of sex-specific expression, it might well be a by-product of strengthening of IeSC driven by an escalating arms race between males and females in the LH population. As sexually antagonistic coevolution in the LH population resulted in an increase in the intensity of IeSC, the signals of IaSC could also evolve to be lower, assuming higher intensities of IeSC correspond to weaker signals of IaSC as suggested by my statistically non-significant results. Therefore, further experimental work aimed at understanding the genetic relationships between traits involved in IaSC and IeSC, as well as their selection gradients under various environments is required. Additionally, it is important to note that while some previous studies reported point estimates of $r_{w,g,mf}$ that were negative, there was considerable uncertainty around these estimates. Furthermore, negative $r_{w,g,mf}$ is not a reliable signal of IaSC (Connallon and Matthews 2019). Overall, $r_{w,g,mf}$ is expected to be negative only when a vast majority of genetic variation is sexually antagonistic in nature. However, genetic variation that is maintained by mutation-selection balance is likely to be sexually concordant. Therefore, our finding of a significantly positive $r_{w,g,mf}$ indicates that sexually concordant fitness variation seems to dominate sexually antagonistic fitness effects.

Using my experimental design, I was also able to obtain estimates of sex-specific heritabilities at the three sex ratios. Consistent with previous studies with similar experimental populations, female heritabilities for adult fitness were higher than male heritabilities (Collet et al. 2016; Ruzicka et al. 2019). Both male and female heritabilities at the female biased sex ratio were considerably lower than male biased or equal sex ratios, suggesting that the rate of adaptation ought to be lower at female biased sex ratio. This is consistent with the findings that experimental evolution at male biased sex ratio leads to rapid sex specific adaptations in reproduction related traits, compared to populations evolving at female biased sex ratio (Nandy et al. 2013a; 2014). An intriguing aspect of my heritability estimates was that they were fairly large. This is likely to be a result of various components of residual fitness variance (that is, fitness variance other than additive genetic variance) being mis-attributed to between-line variance due to some of the shortcomings of my experimental design. In my fitness assays, I expressed entire haploid genomes (barring the "dot" chromosome), in a large number of randomly sampled complementary chromosomes sampled from the LH population. This had two consequences. First, variance

due to epistatic interactions between loci also contributed to between-hemigenome line fitness variance. Second, sampling error in the complementary background in which various hemigenomes of interest were expressed would also inflate the between-hemigenome line fitness variance. Therefore, my estimates of heritabilities likely represent upper bounds for the actual additive genetic variance, rather than heritabilities per se.

An important caveat of my study is that it measures the consequences of altering the intensity of IeSC for one generation to the intensity of IaSC *in the same generation*. This is quite distinct from how signals of IaSC are expected to *evolve* over several generations under either intense IeSC (male biased sex ratio) or weak IeSC (female biased sex ratio). A tractable experimental approach to investigate how signals of IaSC evolve under either high or low intensities of IeSC could involve sampling hemigenomes from populations experimentally evolving at either male biased or female biased sex ratio, and measuring sex-specific fitness for those hemigenomes.

CONCLUSIONS

In conclusion, the key findings of my study are as follows:

1. There was no statistically significant effect of the sex ratio treatment in the signal of IaSC. However, point estimates of the signals of IaSC were lower at male biased sex ratio, relative to female biased sex ratio.

2. In contrast with previous studies, I report significantly positive intersexual genetic correlation for fitness.

3. Both males and females exhibited higher heritabilities for reproductive fitness in male-biased and equal sex-ratio environments as compared to the female-biased sex-ratio.

	npar	logLik	AIC	LRT	Df	p value
<none>	9	-1861.4	3740.9			
(1 | Hemigenome line)	8	-1864	3744	5.114	1	**0.0237**
(1 | Hemigenome line:Sex)	8	-1878	3772	33.147	1	**<0.0001**
(1 | Hemigenome line:Sex.Ratio)	8	-1861.5	3738.9	0.052	1	0.8196
(1 | Hemigenome line:Sex:Sex.Ratio)	8	-1868.2	3752.3	13.479	1	**0.0002**

Table 3.1 ANOVA-like table for random terms in the linear mixed effects model for male and female fitness

A) Using Line Averages				
	Sex Ratio	**Estimate**	**Lower CL**	**Upper CL**
Intersexual genetic correlation for fitness	**Male Biased**	0.3805	0.2992	0.5283
	Equal	0.4027	0.3140	0.5526
	Female Biased	0.2515	0.1198	0.4502
Proportion of sexually antagonistic fitness variation	**Male Biased**	0.3097	0.2358	0.3504
	Equal	0.2986	0.2237	0.3430
	Female Biased	0.3742	0.2749	0.4401
	Pairs of Sex Ratios	**Estimate**	**Lower CL**	**Upper CL**
Genetic correlations for female fitness between pairs of sex ratios	**Male Biased - Female Biased**	0.7688	0.7442	0.8497
	Male Biased - Equal	0.7493	0.7213	0.8368
	Female Biased - Equal	0.8421	0.8403	0.8956
Genetic correlations for male fitness between pairs of sex ratios	**Male Biased - Female Biased**	0.5567	0.4997	0.7262
	Male Biased - Equal	0.6995	0.6755	0.8018
	Female Biased - Equal	0.5415	0.4664	0.7417
B) Using MCMCglmm				
	Sex Ratio	**Estimate**	**Lower CL**	**Upper CL**
Intersexual genetic correlation for fitness	**Male Biased**	0.5056	0.1418	0.7983
	Equal	0.4999	0.1397	0.7787
	Female Biased	0.4462	0.0059	0.8470
Female Heritability	**Male Biased**	0.8702	0.5935	1.1520
	Equal	0.9992	0.7337	1.2696
	Female Biased	0.7385	0.5021	1.0539
Male Heritability	**Male Biased**	0.4788	0.2383	0.7303
	Equal	0.5762	0.3192	0.8637
	Female Biased	0.2229	0.0495	0.4080
	Pairs of Sex Ratios	**Estimate**	**Lower CL**	**Upper CL**
Genetic correlations for female fitness between pairs of sex ratios	**Male Biased - Female Biased**	0.8932	0.6888	0.9996
	Male Biased - Equal	0.8785	0.7477	0.9994
	Female Biased - Equal	0.9536	0.8767	0.9995
Genetic correlations for male fitness between pairs of sex ratios	**Male Biased - Female Biased**	0.8932	0.6888	0.9996
	Male Biased - Equal	0.9438	0.8190	1.0000
	Female Biased - Equal	0.9010	0.7025	0.9997

Table 3.2 The summary of results from A) the analysis using hemigenome line averages and B) the MCMCglmm model. Lower and upper CL represent the limits of 95% confidence intervals.

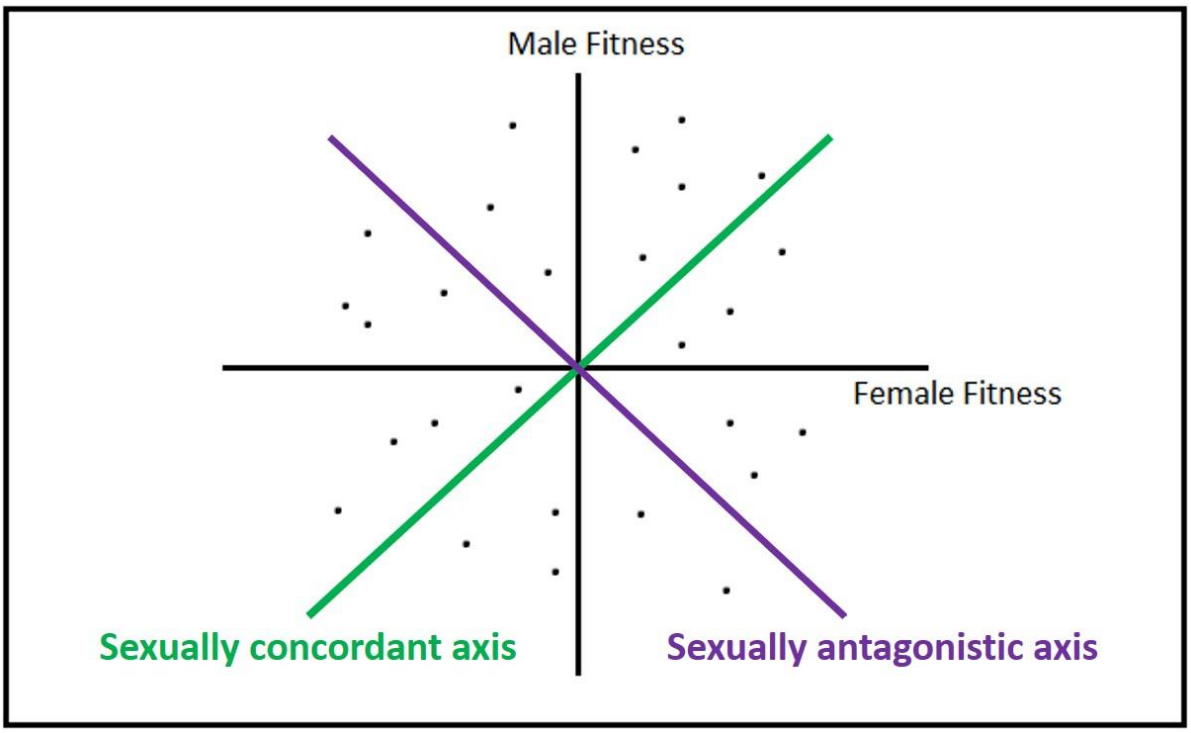

Figure 3.1 A schematic for the axes of sexually antagonistic (purple) and sexually concordant (green) fitness variation. Each black point represents paired male and female fitness scores for a particular genotype. Adapted from Figure 1 of Grieshop and Arnqvist (2018).

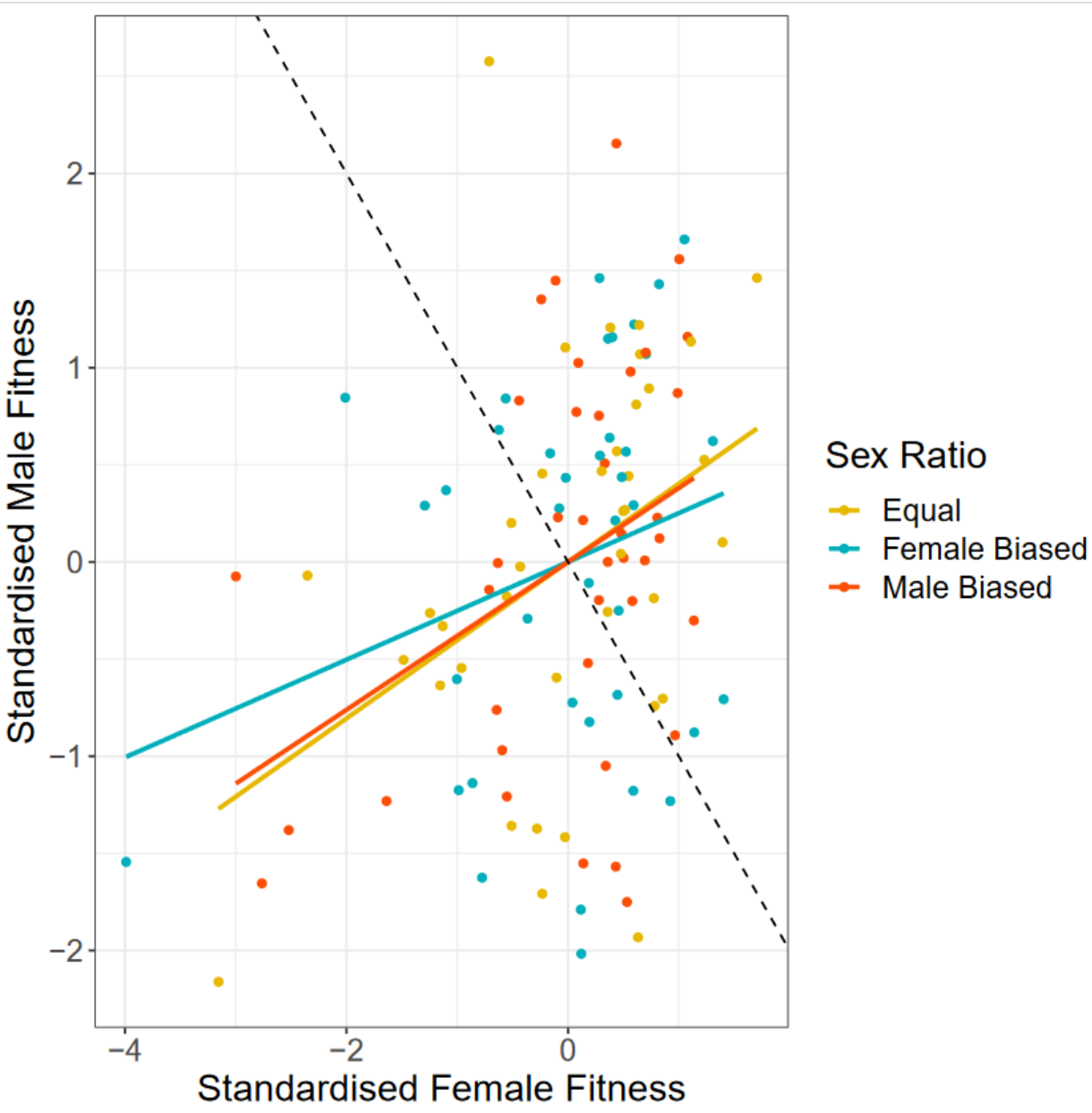

Figure 3.2 Scaled and centred male and female fitnesses for each of the 39 hemigenome lines for equal sex-ratio (yellow), female-biased sex ratio (blue) and male-biased sex-ratio (red). The solid lines represent the least-squared regression lines for each of the three sex-ratios. The dashed line represents the axis of sexually antagonistic fitness variation with male-beneficial, female detrimental genptypes to the top-left and female-beneficial, male detrimental genotypees to the bottom-right.

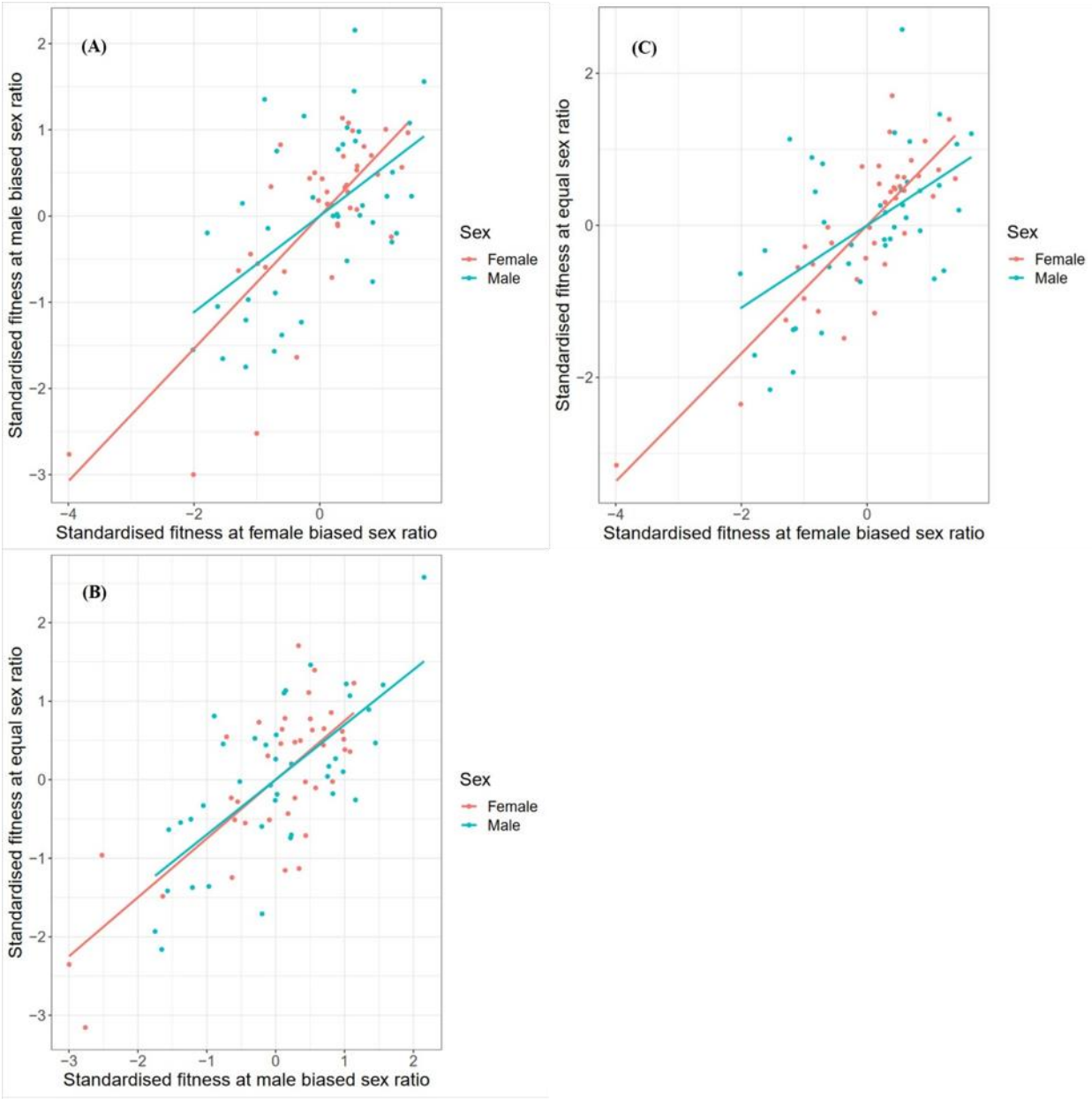

Figure 3.3 Scatterplots showing standardised male and female fitnesses for various hemigenome lines between (A) male biased and female biased sex ratios, (B) equal and male biased sex ratios, and (C) equal and female biased sex ratios. Blue represents data for males, and red represents data for females. The solid lines represent least-squared regression lines.

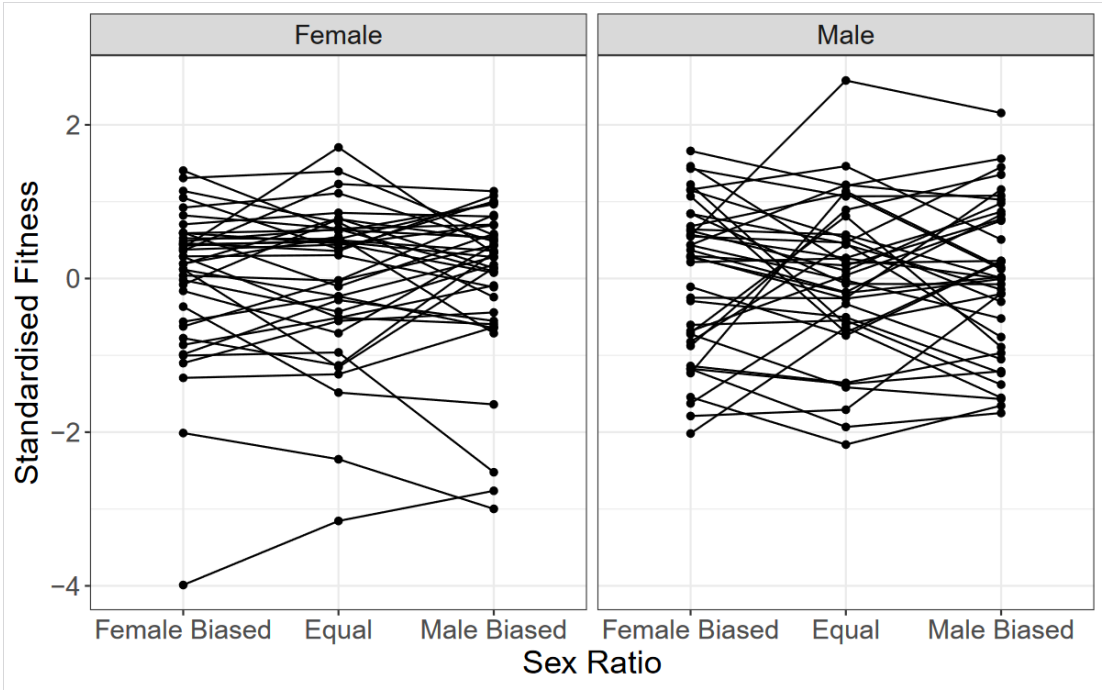

Figure 3.4 Interaction plots showing standardized fitnesses for various hemigenome lines at female biased, equal, and male biased sex ratios for females and males. Points connected by a line represent a hemigenome line.

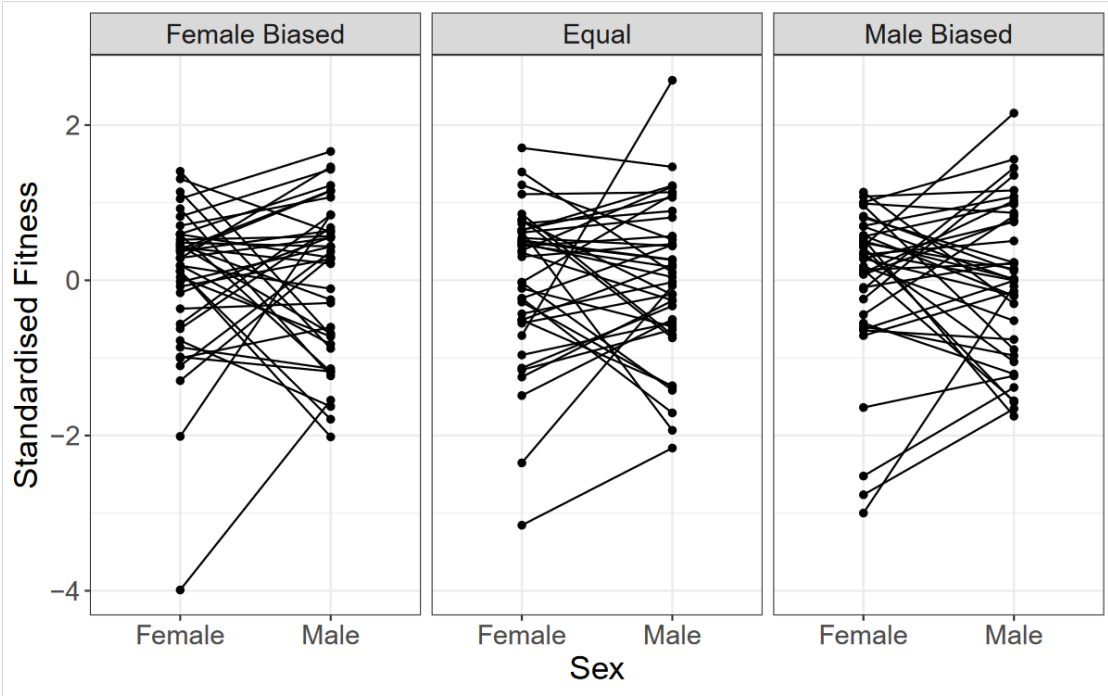

Figure 3.5 Interaction plots showing standardized fitness for various hemigenome lines for females and males, at female biased, equal, and male biased sex ratios. Points connected by a line represent a hemigenome line.

Chapter 4

Sex-specific traits and the interaction between Interlocus and Intralocus Sexual Conflict

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INTRODUCTION

In many species, the investment females make per bout of reproduction is considerably higher than the investment made by males (Trivers 1972), a pattern thought to be a product of anisogamy (Parker 1982) (but see Hayward and Gillooly (2011); Iyer et al. (2020); Kokko and Jennions (2003)). A consequence of this is the disparity between males and females for the relationship between the number of matings and fitness. With less investment per bout of reproduction, male fitness is thought to increase linearly with number of matings, while female fitness is thought to have an intermediate optimum number of matings, imposed by an upper bound to the number of eggs they can produce. This pattern was experimentally confirmed by Bateman (1948). While Bateman's (1948) findings have been questioned from the scientific (Kokko et al. 2012; Roughgarden 2009; Roughgarden 2015) as well as philosophical (Hoquet 2020a; 2020b) point of view, patterns consistent with them abound in the nature (Janicke et al. 2016; Singh and Punzalan 2018). It has also been pointed out that irrespective of the validity of Bateman's (1948) study, the usefulness of Bateman's gradients as an empirical tool cannot be denied (Morimoto 2020).

Theoretical studies predict that when males and females have distinct fitness optima for mating rates, it can trigger sexual conflict (sensu Parker (1979)) characterised by sexually antagonistic coevolution (Gavrilets et al. 2001; Rowe et al. 2005). Male and female traits that determine the average population mating rates (sometimes called "persistence" and "resistance" traits, respectively) are modelled to be sex-limited in their effects. Thus, sexual conflict over mating rates, in its theoretical formalism, is a conflict between one set of loci limited to males, and a different set of loci limited to females. Thus, the sexual conflict over mating rates is an "interlocus" sexual conflict (IeSC). There is considerable evidence of IeSC over mating rates, but few examples are more dramatic than that of water striders, many species of which exhibit an intense "pre-mating struggle". In one of the species of water striders, males have evolved specialised structures on their antennae that fit at

specific positions on the female's head enabling males to grasp females during copulation (Khila et al. 2012). IeSC can also arise over other aspects of reproductive interactions between males and females such as the interactions between components of male ejaculate and the female reproductive tract (Sirot et al. 2015), parental care (McNamara and Wolf 2015), and even optimal sex ratios in haplodiploid organisms (Macke et al. 2014).

While most of the early modelling work on IeSC treated resistance and persistence related traits to be sex-limited in their effects, some recent studies have explored the evolutionary consequences of relaxing this assumption. This is particularly relevant to the question of whether IeSC interacts with the other type of sexual conflict: Intralocus Sexual Conflict (IaSC). IaSC is usually modelled as a conflict over traits expressed in both sexes but subject to sexually antagonistic selection (see Chapter 1 for a detailed overview of IaSC). Pennell and Morrow (2013) argued that if resistance and persistence traits are not entirely sex-limited in their effects, but have pleiotropic fitness effects when expressed in the opposite sex, IaSC could ensue over resistance and persistence traits as well. As an example, Pennell et al. (2016) suggested that females could evolve greater resistance to male courtship by reducing sensitivity to male sexual signals. This could potentially involve changes to the sensory apparatus, which could have negative effects on male fitness, triggering IaSC. They further modelled the evolutionary dynamics of such a system, and showed that if resistance and persistence traits have negative fitness consequences when expressed in the opposite sex, it can have a damping effect on sexually antagonistic coevolution. A second way in which IaSC and IeSC can interact is if resistance and persistence are genetically correlated. Assortative mating arising if the most resistant females mate more frequently with the most persistence males, could lead to such correlations building up in the populations. Experimentally increasing the strength of IeSC, in other words increasing the strength of selection acting on resistance and persistence traits, would lead to stronger sexually concordant selection. All else being equal, this would translate to a weaker signal of IaSC at higher intensities of IeSC.

Studies aiming to experimentally alter the intensity of IeSC have taken one of two different approaches. Some studies have experimentally enforced monogamy and compared their evolution to promiscuous populations (Crudgington et al. 2010; Demont et al. 2014; Gay et al. 2011; Holland and Rice 1999; Hosken et al. 2001; Tilszer et al. 2006). On the other hand, some studies have employed experimentally altered adult sex ratios (Michalczyk et al. 2011; Nandy et al. 2013a; 2014 ; Wigby and Chapman 2004) with the intensity of IeSC

thought to be higher at male biased sex ratios (Emlen and Oring 1977). While there is substantial evidence that male biased sex ratios correspond to increased strength of sexual selection and IeSC, that may not always be the case as some theoretical studies have demonstrated (Klug et al. 2010).

In this chapter, I first validated whether the sex ratio treatments I used in Chapter 3 actually translated to changes in the intensity of sexual selection and IeSC along expected lines (i.e., stronger IeSC at male biased sex ratio). This was done by measuring three different quantities at male biased, equal and female biased sex ratios: (1) additive genetic variance for relative fitness for males and females (or the opportunity for sexual selection), (2) selection gradients on male fitness-related traits, and (3) fecundity of baseline females. If IeSC and sexual selection is indeed stronger at the male biased sex ratio, one would expect the additive genetic variance for relative male fitness and selection gradients on male fitness-related traits to be higher at the male biased sex ratio. On the other hand, stronger IeSC at male biased sex ratio would translate to greater male-induced mate harm experienced by females, and therefore, reduced fecundity at the male biased sex ratio. Next, I investigated whether IaSC and IeSC can interact via genetic correlations between resistance and persistence traits, or correlations between resistance/persistence and the fitness of the opposite sex. The signs of these correlations would depend on the nature of the interaction between IaSC and IeSC. If these two kinds of conflict reinforce each other, one would expect resistance/persistence traits to have negative fitness consequences when expressed in the opposite sex. Lastly, this data also allowed me to address whether there are genetic trade-offs between various components of male reproductive success.

METHODS

Generating experimental flies

I used the panel of hemigenomes sampled in Chapter 2 for experiments described below. I also made use of the fitness data for these lines generated in Chapter 3. Chapter 2 describes the detailed protocol for generating focal males and females expressing target hemigenomes in a background randomly sampled from the LH population to generate hemiclonal flies. To express target hemigenomes in female, I crossed males from each hemigenome line that were heterozygous for the target hemigenome and the translocation between chromosome II and chromosome III with virgin LH females. The female progeny from these crosses were either heterozygous for the translocation and a random set of

chromosomes from the LH population (brown eyed due to the brown dominant marker on the translocation) or expressed the target hemigenome in a random LH background (red eyed). I discarded the brown eyed female progeny, and collected the red eyed female progeny as virgin (i.e., within 6 h of eclosion) using light CO_2 anaesthesia. The protocol for generating experimental males was similar, except I crossed males from each hemigenome line that were heterozygous for the target hemigenome and the translocation with virgin DxLH females. The experiments described below also used flies from the LHst population. In order to generate these flies, I collected eggs from the LHst population at a density of 150 eggs per vial containing 8-10 ml food. I collected LHst males and females as virgin with the help of mild CO_2 anaesthesia. Additionally, I ensured that at the time of the assays described below, all experimental flies were 2-3 day old as adults, i.e., they were age matched.

I measured the contribution of each hemigenome line towards mating related traits in both sexes as well as sperm competitive ability in males.

Mating traits in females

First mating: I collected virgin focal females as described above in groups of 8 females per vials. On the 12^{th} day post egg collection, I combined these females individually with virgin LHst males in a food vial. For every pair, I measured the time the pair took to initiate copulation after being combined in the vial (i.e., mating latency) and the time for which the pair remained in copula (i.e., copulation duration). I repeated this experiment thrice on separate days. In each replicate, I measured the mating latency and copulation duration for 10 virgin females from each hemigenome line. Pairs that did not mate, even after 60 minutes post combination, were discarded.

Second mating: In *D. melanogaster*, while latency to first mating is usually a few minutes, singly mated females can take up to several hours to mate again. After the first mating was over, I discarded LHst males. In the same vials, I then introduced fresh virgin LHst males. I observed each vial until the focal females mated with the second LHst males, and recorded the time interval between the introduction of second males and the initiation of mating (hereafter referred to as "female remating latency"). I continued these observations for 24 h, by which time around 80% females had remated. The females that did not remate by this time were excluded from the analyses.

Mating rate: Separately, I also performed a female mating rate assay. I made mating rate measurements at male biased and females biased sex ratios in an assay that was also used to measure locomotory activity (Chapter 5a). I generated focal females as described above, and collected them as virgin in groups of 8 per vial. I also collected LHst males as virgin in groups of 8 per vial. On the 12th day post egg collection, between 6 pm and 7 pm, I combined focal females and LHst males in appropriate numbers (8 females: 24 males for male biased sex ratio, and 24 females:8 males for female biased sex ratio) in food vials supplemented with 100 μL live yeast paste. As in Chapter 3, the concentration of the yeast paste was such that the per female supplementary yeast availability in each vial was 0.47 mg. For every hemigenome line, I set up one vial where the sex ratio was male biased, and another where it was female biased. Beginning from 11 pm on the 12th day post egg collection, I recorded the number of mating pairs in each vial, once every two hours till 5 pm in the 14th day. Note that this period is where most of the reproductive interactions that determine fitness occur in the LH population.

Mating traits in males

First mating: This assay was a mirror image of the female first mating assay. Briefly, I combined virgin focal males with virgin LHst females individually in food vials. I then recorded the mating latency and the copulation duration in each vial. As before, pairs that failed to mate within 60 minutes were discarded. I repeated this experiment thrice on separate days. In each replicate, I measured the mating latency and copulation duration for 10 virgin males from each hemigenome line.

Second mating: First, I combined virgin males and females from the LHst population individually in food vials. I ensured that a single mating happened in each vial. Vials where the pair did not mate within 60 minutes were discarded. In the vials where mating did occur, I aspirated out the males once the mating was complete. Then I introduced virgin focal males in each vial, and recorded the time they took to initiate copulation with the once-mated LHst females. For each hemigenome line I set up 10 vials. I observed these vials continuously for 24 h, by which time second matings had occurred in about 80% vials. I discarded the remaining vials. I repeated this assay thrice on different days.

Mating rate: The male mating rate assay was an exact mirror image of the female mating rate assay. On the 12th day post egg collection, I combined virgin focal males with virgin LHst females, in appropriate numbers to obtain male biased and female biased sex ratios

used for the female mating rate assay in food vials supplemented with a live yeast paste. I recorded the number of mating pairs between 11 PM on the 12^{th} day and 5 PM on the 14^{th} day every two hours. As in the female assay, I had one vial for each hemigenome line at each of the two sex ratios, and I replicated this experiment thrice.

Fecundity of LHst females held with focal males

In order to explore the mate harming ability of males carrying various target hemigenomes, I measured the fecundity of baseline females held with focal males at male biased and female biased sex ratios. At the end of the male mating rate assay (see above), I transferred four females from each vial to fresh food vials. I allowed these females to lay eggs for 18 h, after which they were discarded. I then counted the eggs laid by these females in each vial.

Male sperm competitive ability

Sperm defence ability (P1): Sperm defence ability (P1) usually measures the ability of the focal male's ejaculate to minimise displacement by the ejaculate of a male that mates subsequently. In order to measure P1, I used the focal males from the first mating assay described above. After the focal males had mated with virgin LHst females, I aspirated out the focal males. I then introduced virgin LHst males to those vials. I then observed each vial continuously until the pair started mating. Pairs that did not mate for 24 h after introduction of the 2^{nd} males were discarded. I then discarded the males and transferred the LHst females individually to test tubes (diameter 0.5 mm, length 10 mm) containing food. I allowed these females to lay eggs in the test tubes for 18 h, after which I discarded the females and incubated the test tubes under standard conditions. After 10-11 days, when all the progeny from each test tube had eclosed, I froze the test tubes at -20^0 C. I then scored the progeny from each test tube for their eye colour. Notice that the focal males were red eyed. As the scarlet eye colour mutation is recessive, the progeny sired by the focal males were red eyed, while the progeny sired by the LHst males were scarlet eyed. I calculated the proportion of red eyed progeny from each vial and used it as a measure of P1. I repeated the P1 assay thrice; within each assay there were 7-10 vials for each hemigenome line, contingent on how many rematings occurred.

Sperm offence ability (P2): Sperm offence ability (P2) is the focal male's ability to displace the sperm already present in the female's reproductive tract. I used the twice-mated LHst females from the male second mating assay to measure P2 of the focal males. Notice that

these females had mated with LHst males first, followed by the focal males. After the second mating, I discarded the second males and transferred the females to test tubes (diameter 0.5 mm, length 10 mm) containing food. After giving the females 18 h to lay eggs, I discarded them and incubated the eggs under standard conditions. As in the P1 assay, I scored the progeny emerging from each test tube for their eye colour and used the proportion of red-eyed progeny from each test tube as the measure of P2. There were three independent replicates of the P2 assay, and for each assay I had 8-10 replicate vials for each hemigenome line, depending on how many rematings were observed.

STATISTICAL ANALYSIS

A. Sex-specific additive genetic variance at male biased, equal and female biased sex ratios

I used the sex-specific fitness data at male biased, equal, and female biased sex ratios obtained in Chapter 3. I used competitive fertilisation success as the measure of male fitness, and fecundity post competition for limiting amounts of live yeast as the measure of female fitness. I first calculated the average fitness of each hemigenome line as males and as females at each of the three sex ratios. I calculated the relative fitness of each hemigenome line (in each sex and sex ratio) by dividing the sex- and sex ratio-specific fitness of each hemigenome line by the mean fitness at that combination of sex and sex ratio. I then calculated the variance in these line averages of relative fitness for each combination of sex and sex ratio. I then tested, separately for males and females, whether the variances were different between male biased, equal and female biased sex ratios using the Levene's test.

Note that these estimates measure the *total* additive genetic variance for fitness, separately for each sex and sex ratio. Therefore, they are quite different from the sex- and sex ratio-specific heritabilities reported in Chapter 3, which were equivalent to the *proportion* of the total variance that was due to additive genetic effects.

B. Fecundity of LHst female at male biased and female biased sex ratio

Using the fecundity of LHst females measured in the male mating rate assay, I fit the following linear mixed effects model using the R packages "lme4" (Bates et al. 2022) and "lmerTest" (Kuznetsova et al. 2020):

Fecundity ~ Sex Ratio + (1|Hemigenome line) + (1|Replicate)

To investigate if increasing male fitness translated to greater mate harm to females at male biased sex ratio, I calculated the line averages for fecundity of baseline females when held with focal males at the two sex ratios. Separately for each sex ratio, I then regressed line averages for male fitness with line averages for fecundity of baseline females.

C. Genetic architecture of reproduction related traits

First, using the R packages "lme4" and "lmerTest" I fitted the following linear mixed effects model, separately for each trait and each sex (latency to first mating, copulation duration, remating latency, mating rates at male biased or female biased sex ratio, fecundity of baseline female held with focal males at male biased or female biased sex ratios, P1, P2):

Y ~ (1|Hemigenome line) + (1|Replicate)

To formally investigate the sex-specific genetic architecture of resistance and persistence traits, using the R package "MCMCglmm" (Hadfield 2010), I fit the following Bayesian linear mixed models separately for various definitions of resistance and persistence (i.e., latency to first mating, copulation duration, remating latency):

$Y_{ijkl} \sim S_i + L_{ij} + R_{ik} + \varepsilon_{ijkl}$, Y_{ijkl} where is the standardised trait value of l^{th} fly, of replicate assay k, hemigenome line j and sex i. S_i is the fixed effect of sex, L_{ij} models the random sex-specific effect of hemigenome lines, R_{ik} describes the random sex-specific effect of replicate assay, and ε_{ijkl} describes the residuals. L_{ij} is a modelled to follow a multivariate normal distribution with a mean 0, and variances and covariances given by the sex-specific line variances and intersexual line covariances for the trait. R_{ik} and ε_{ijkl} are modelled to follow normal distributions with means 0, and variances given by sex-specific block variance and sex-specific residual variance, respectively. For each model, I ran the simulation for 100000 iterations, out of which the first 25000 were discarded as "burn-in". In the next 75000 iterations every 50^{th} iteration was sampled to create posterior distributions of the quantities of interest.

For each trait, I obtained the posterior distributions for the sex-specific heritabilities, and the intersesxual genetic correlation (r_{mf}). I used the following expressions:

$$Heritability = \frac{2 \times Line\ variance}{(Line\ variance + block\ variance + residual\ variance)}$$

$$r_{mf} = \frac{Intersexual\ genetic\ covariance}{\sqrt{Line\ variance\ in\ females} \times \sqrt{Line\ variance\ in\ males}}$$

I multiplied the line variance in the denominator by 2, because hemiclonal individuals representing a hemigenome line only share one half of their genomes.

I also fitted separate Bayesian linear mixed models for sperm competitive ability (P1 and P2) and the fecundity of baseline females at male biased and female biased sex ratio. These models were identical to the model described above, except sex was replaced by trait (P1 or P2) in the model for sperm competitive ability and by sex ratio (female biased or male biased) in the model for fecundity of baseline females held with focal males.

D. Linear and quadratic selection gradient.

In order to calculate linear selection gradients, I fit the following linear models, separately for each trait and each sex ratio:

Relative fitness ~ Trait

In order to calculate the quadratic selection gradients, I fit the following linear models, separately for each trait and each sex ratio:

Relative fitness ~ Trait + $(\text{Trait})^2$

In order to calculate the quadratic selection gradient, I multiplied the coefficient of the $(\text{Trait})^2$ term by 2 following Stinchcombe et al. (2008).

E. Genetic correlations between reproduction related traits and fitness in the opposite sex

In order to investigate if resistance and/or persistence related traits have pleiotropic fitness effects when expressed in the opposite sex thereby influencing patterns IaSC, I calculated the Pearson's product-moment correlation between line averages for relative female fitness and line averages for male traits (latency to first mating, copulation duration, remating latency, P1, P2, mating rate and fecundity of baseline females held with focal males), as well as, the Pearson's product-moment correlation between line averages for relative male fitness and the line averages for female traits (latency to first mating, copulation duration, remating latency, mating rate). Note that *lower* mating latency corresponds to *higher* persistence in males. Therefore, a negative genetic correlation between male mating latency

and female fitness is equivalent to a *positive* genetic correlation between male persistence and female fitness.

F. Genetic correlations between sperm defence or sperm offence ability with male mating traits

In order to investigate if there were any genetic trade-offs between pre- and post-copulatory traits in males, I measured the Pearson's product-moment correlation between line averages for P1 (or P2) and line averages for mating related traits such as latency to first mating, latency to second mating, copulation duration, and mating rates.

RESULTS

A. Sex-specific additive genetic variance at male biased, equal and female biased sex ratios

I measured line variances for sex- and sex ratio-specific relative fitnesses. If the male biased sex ratio corresponds to stronger IeSC, the line variance for relative fitness ought to be higher at the male biased sex ratio relative to the other sex ratios. Consistent with this prediction, Levene's revealed that there was a statistically significant effect of sex ratio treatment on the line variance in relative fitness for males ($F = 4.1982$, numerator DF = 2, denominator DF = 114, p-value = 0.0174), with the line variances for relative male fitness being the highest at male biased sex ratio, followed by equal sex ratio, and lowest at female biased sex ratio (Figure 4.1). Line variances for male relative fitness at male biased equal and female biased sex ratio were 0.2926 (95% CI = [0.1954, 0.4859]), 0.1591 (95% CI = [0.1062, 0.2642]), and 0.1010 (95% CI = [0.0674, 0.1678]) respectively (Figure 4,1). For females, I did not detect any effect of the sex ratio treatment on the line variances for relative fitness (Levene's test, $F = 0.9396$, numerator DF = 2, denominator DF = 114, p-value = 0.3938). While the line variance for relative female fitness was considerably lower than males, it followed the same relative trend among sex ratios. The line variances for females at male biased, equal, and female biased sex ratio were 0.0532 (95% CI = [0.0355, 0.0884]), 0.0321 (95% CI = [0.0214, 0.0533]), and 0.0254 (95% CI = [0.0170, 0.0422]), respectively (Figure 4.1).

B. Fecundity of LHst female at male biased and female biased sex ratio

If IeSC is stronger at the male biased sex ratio, female fecundity at the male biased sex ratio ought to be lower as a consequence of greater male induced mate harm. Consistent with

this, the linear mixed effects models found a statistically significant effect of sex ratio ($p < 0.0001$) (Table 4.1), with female fecundity being lower at the male biased sex ratio compared to female biased sex ratio (Figure 4.2).

C. Genetic architecture of reproduction related traits

The linear mixed models found a statistically significant effect of hemigenome line in males for latency to first mating, latency to second mating, copulation duration, fecundity of baseline females held with focal males at female biased sex ratio, P1, and P2, but not for mating rates at both sex ratios, and fecundity of baseline females held with focal males at male biased sex ratio (Table 4.1). In females, there was a statistically significant effect of hemigenome line for latency to first mating, remating latency, but not for copulation duration, or mating rates at either of the sex ratios (Table 4.2).

The estimates of heritabilities and genetic correlations for various traits obtained in the Bayesian linear mixed models are summarised in Table 4.3. Interestingly the genetic correlation between the sexes were not significantly different from 0 for latency to first mating (-0.0176, 95% CI = [-0.5443, 0.5216]), copulation duration (0.2824, 95% CI = [-0.4522, 0.9772]), remating latency (0.0021, 95% CI = [-0.4206, 0.4543]) and mating rates at male biased (0.2585, 95% CI = [-0.5404, 0.9651]) or female biased (-0.0385, 95% = [-0.9403, 0.8272]) sex ratios (Figure 4.3). Additionally, I found that the genetic correlation between P1 and P2 was significantly positive (0.7868, 95% CI = [0.5281, 0.9975]), while the genetic correlation between fecundity of baseline females held with focal males at male biased and female biased sex ratios was negative, but not significantly different from 0 (-0.4647, 95% CI = [-0.9960, 0.2499]) (Figure 4.4).

D. Linear and quadratic selection gradients

In males, I found statistically significant linear selection gradients on latency to first mating, copulation duration, remating latency, P1, P2, and fecundity of baseline females held with focal males at female biased sex ratio. Typically, linear selection gradients were steeper at male biased sex ratio, followed by equal and then by female biased sex ratio (Table 4.4A, Figure 4.5), suggesting that sexual selection was indeed stronger at the male biased sex ratio. Mating early, both with virgin females as well as singly mated females, was genetically correlated with greater male fitness. Mating for a longer duration was genetically correlated with greater fitness. However, this trend was not statistically significant. Greater sperm competitive ability was positively genetically correlated with

male fitness. There was a significantly positive genetic correlation between male fitness and the fecundity of baseline females held with focal males at female biased sex ratio. At the male biased sex ratio, this relationship was negative, although not significantly so. There was no statistically significant genetic correlation between male fitness and mating rates (Figure 4.6). The quadratic selection gradients in males were significantly different from 0 in certain cases (Table 4.4B). For example, at equal and male biased sex ratios there was disruptive selection on latency to first mating. At female biased sex ratio, there also appeared to be statistically significant stabilising selection on P1.

In females, none of the selection gradients were significantly different from 0, except for copulation duration at equal and male biased sex ratio (Table 4.5A, Figure 4.5-4.6). Shorter copulations were associated with greater female fitness. Similarly, none of the quadratic selection gradients on females were significantly different from 0 (Table 4.5B).

E. Genetic correlations between reproduction related traits and fitness in the opposite sex

One of the ways in which IaSC and IeSC can interact if traits involved in IeSC have pleiotropic consequences when expressed in the opposite sex. If IaSC and IeSC reinforce each other these consequences ought to be negative. Male latency to first mating was negatively genetically correlated with female fitness at female biased and equal sex ratios. Since lower male mating latency corresponds greater male persistence, this result translates to a *positive* genetic correlation between male persistence and female fitness. P1 and P2 were both positively genetically correlated with female fitness at female biased sex ratio (Table 4.6A, Figure 4.7). Female mating rate was negatively genetically correlated with male fitness at female biased sex ratio (Table 4.6B, Figure 4.8).

F. Genetic correlations between sperm defence or sperm offence ability with male mating traits

The genetic correlations between P1 or P2 and various male mating traits are summarised in Table 4.7. Both P1 and P2 were significantly negatively genetically correlated with male latency to first mating (Figure 4.9A-B) as well as remating latency (Figure 4.9 E-F). Genetic correlations between P1 or P2 and copulation were positive (Figure 4.9 C-D), but not significantly different from 0. Genetic correlations between P1 or P2 and mating rate were positive at male biased sex ratio (Figure 4.9G), and negative at female biased sex ratio

(Figure 4.9H), although neither of these correlations were significantly different from 0 (Table 4.7).

DISCUSSION

In this chapter, I first investigated whether the intensity of IeSC and sexual selection was stronger at male biased sex ratio, compared to female biased sex ratio. Next, I asked whether genetic correlations between resistance and persistence traits, or genetic correlations between resistance/persistence and fitness of the opposite sex were driving patterns of IaSC in the population. Lastly, I asked whether there were any genetic trade-offs between male reproduction related traits. Below, I discuss my principal findings.

Evidence of stronger IeSC and sexual selection at male biased sex ratio

I found compelling evidence that male biased sex ratio corresponded to stronger IeSC and sexual selection. First, additive genetic variance for relative fitness for males (i.e., opportunity for selection) was significantly higher at male biased sex ratio, relative to female biased sex ratio. The fact that there was stronger selection operating at male biased sex ratio was confirmed by the steeper linear selection gradients on reproduction related traits, particularly male traits. Furthermore, there appeared to be a strong dissonance in male and female reproductive fitness at male biased sex ratio. First, females at male biased sex ratio laid substantially fewer eggs, compared to their counterparts held at female biased sex ratio, suggesting that females were exposed to male induced mate harm at male biased sex ratio. Second, the genetic correlation between male fitness and the fecundity of baseline females held with focal males was positive at female biased sex ratio, but negative (although not significantly so) at male biased sex ratio. This suggests that at male biased sex ratio, genotypes that were the fittest as males were also more harmful towards females, a clear signature of IeSC. Overall, these results are in tune with the vast body of experimental evolution studies, that found rapid evolution of sexually selected and IeSC related traits at male biased sex ratio (Dore et al. 2021; Maggu et al. 2022; McNamara et al. 2020; Michalczyk et al. 2011; Nandy et al. 2013a; 2014; Sepil et al. 2021; Wigby and Chapman 2004;). Another interesting result was the statistically non-significant negative genetic correlation between fecundity of baseline females held with focal males at female biased and male biased sex ratios. This result was somewhat similar to the negative relationship Filice and Long (2016) detected between the fecundity of females exposed to focal males for a short duration and the degree of mate harm (i.e. fecundity reduction

between short exposure and long exposure treatments) caused by focal males. These findings seem to suggest that in *D. melanogaster* male genotypes that are more harmful to their mates in an environment where IeSC is intense (e.g., male biased sex ratio, or continuous exposure treatment) also provide direct fitness benefits to females in an environment where IeSC is benign (e.g., female biased sex ratio, or short exposure treatment). This has some interesting implications to female mate choice. At female biased sex ratio, a female would accrue considerable direct benefits of mating with the "fitter" males. However, at the male biased sex ratio, exposure to fitter males would extract a substantial cost on female fitness. Therefore, females evolving under female biased sex ratio should evolve a stronger mating bias towards fitter males. Chechi et al. (2022) evolved replicate populations of *D. melanogaster* under either male biased (M) or female biased (F) sex ratios, and measured the mating success of M and F males when in direct competition for either M, F, or ancestral females. Their findings were, however, not consistent with the prediction outlined above. They reported that M males consistently exhibited greater mating success than F males, irrespective of the female they were competing for. However, variation in mating success is a function of both differences in the competitive abilities of competing males and female choice. The experimental design used by Chechi et al. (2022) does not distinguish between these two possibilities.

No genetic correlations between resistance and persistence

My analyses detected substantial additive genetic variation in both males and females for latency to first mating, remating latency, but not for mating rates. There was also substantial additive genetic variation for copulation duration in males, as well as sperm defence (P1) and sperm offence ability (P2), but not for copulation duration in females. Additionally, I could not detect any statistically significant genetic correlations between resistance and persistence, regardless of how the two were measured. This was consistent with the findings of Rice et al. (2005) who failed to detect any genetic correlation between remating rates in their population.

Positive genetic correlations between persistence and female fitness

I found a negative genetic correlation between female fitness and male latency to first mating as well as remating latency. Since early mating is equivalent to greater male persistence, this translates a *positive* relationship between male persistence and female fitness. However, this relationship was statistically significant only at female biased and

equal sex ratios for latency to first mating. Similarly, both P1 and P2 were positively genetically correlated with female fitness, but only at female biased sex ratio. Interestingly, at male biased sex ratio, where these traits were under stronger selection in males (Figure 4.5), I did not detect any statistically significant genetic correlations between male traits and female fitness. Therefore, it is not entirely clear whether these correlations drive patterns of IaSC I detected in Chapter 3.

IeSC over copulation duration at male biased sex ratio

I found that the linear selection gradients on male copulation duration were positive, being the steepest at male biased sex ratio. On the other hand, the linear selection gradients on female copulation duration were negative, with the selection gradient at male biased sex ratio being the steepest. These results, coupled with the fact that the intersexual genetic correlation for copulation duration was not different from 0, point towards unmistakable signs of IeSC over the duration of copulation. Typically, IeSC over copulation duration has been reported in insect species with traumatic insemination such as *Callosobruchus maculates* (Crudgington and Siva-Jothy 2000). There is some evidence of sexual conflict over copulation duration in *Drosophila montana* (Mazzi et al. 2009). To the best of my knowledge, this is one of the few reports of IeSC over copulation duration in *D. melanogaster*. While longer copulations are generally correlated with male fitness (Bretman et al. 2009; Maggu et al. 2022; Nandy and Prasad 2011), it was interesting that females benefited from shorter copulations. While typically, *D. melanogaster* copulations last around 15-20 minutes, sperm transfer is completed by midway in the copulation (Gilchrist and Partridge 2000). Therefore, effects of longer copulation are likely to be due to increased transfer of accessory gland proteins (ACPs) (Sirot et al. 2015; Wolfner 1997).

No genetic trade-offs between pre- and post-copulatory male traits

I found strongly positive genetic correlations between both P1 and P2 with male mating ability (measured in terms of latency to first mating, as well as remating latency). Genotypes with greater sperm competitive abilities also, on average, mated faster as males. Since both pre- and post-copulatory traits are energetically costly (Allen and Levinton 2007; Dewsbury 1982; Emlen 2001), theoretical work predicts that the two should exhibit trade-offs between them (Parker et al. 2013). However, recent work has shown that the nature of covariance between pre- and post-copulatory traits should depend on the marginal fitness benefits gained upon increasing the investment in these traits. In a

macroevolutionary comparative study, Lüpold et al. (2014) found that the covariance between pre- and post-copulatory male traits was positive for taxa with scramble competition, but negative for those with contest competition. This suggests that mating interactions in *D. melanogaster* are largely driven by scramble competition.

CONCLUSION

My findings suggest that the strength of IeSC and sexual selection is higher at male biased sex ratio, compared to female biased sex ratio. I found no evidence of genetic correlations between resistance and persistence. Some male reproduction related traits were positively genetically correlated with female fitness, but not at male biased sex ratio. There was evidence of IeSC over copulation duration at male biased sex ratio. Male pre- and post-copulatory traits were positively genetically correlated.

Linear mixed models for male traits						
(A) Latency to first mating						
	npar	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-3783.1	7574.2			
(1|Hemigenome line)	3	-3806.7	7619.4	47.232	1	**<0.0001**
(1|Replicate)	3	-3783.1	7572.2	0.005	1	0.9421
(B) Copulation duration						
	npar	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-2931.3	5870.5			
(1|Hemigenome line)	3	-2962.2	5930.5	61.951	1	**<0.0001**
(1|Replicate)	3	-2950.4	5906.8	38.336	1	**<0.0001**
(C) Remating latency						
	npar	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-6494.5	12997			
(1|Hemigenome line)	3	-6528.4	13063	67.793	1	**<0.0001**
(1|Replicate)	3	-6494.7	12995	0.409	1	0.5225
(D) Mating rates (female biased sex ratio)						
	npar	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-238.79	485.57			
(1|Hemigenome line)	3	-239.3	484.6	1.0312	1	0.3099
(1|Replicate)	3	-239.29	484.58	1.0073	1	0.3155
(E) Mating rates (male biased sex ratio)						
	npar	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-201.49	410.98			
(1|Hemigenome line)	3	-202.66	411.31	2.3333	1	0.1266
(1|Replicate)	3	-202.08	410.17	1.1912	1	0.2751
(F) Fecundity of baseline females (female biased sex ratio)						
	npar	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-394.93	797.86			
(1|Hemigenome line)	3	-398.23	802.46	6.5989	1	**0.0102**
(1|Replicate)	3	-398.72	803.44	7.5862	1	**0.0060**
(G) Fecundity of baseline females (male biased sex ratio)						
	npar	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-398.26	804.53			
(1|Hemigenome line)	3	-398.42	802.84	0.309	1	0.5785
(1|Replicate)	3	-415.51	837.01	34.485	1	**<0.0001**
(H) Sperm defence ability (P1)						
	npar	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-59.776	127.55			
(1|Hemigenome line)	3	-72.127	150.25	24.7012	1	**<0.0001**
(1|Replicate)	3	-60.317	126.64	1.0827	1	0.2981
(I) Sperm offence ability (P2)						
	npar	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-263.23	534.45			
(1|Hemigenome line)	3	-287.93	581.86	49.411	1	**<0.0001**
(1|Replicate)	3	-263.23	532.45	0	1	1

Table 4.1 Outputs of linear mixed effects models for male traits showing effects of hemigenome line

Linear mixed models for female traits						
(A) Latency to first mating						
	npar	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-3791.5	7591			
(1|Hemigenome line)	3	-3794.9	7595.9	6.8784	1	**0.0087**
(1|Replicate)	3	-3800.3	7606.6	17.5498	1	<0.0001
(B) Copulation duration						
	npar	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-3315.3	6638.5			
(1|Hemigenome line)	3	-3315.7	6637.5	0.9664	1	0.326
(1|Replicate)	3	-3324.7	6655.4	18.8664	1	**<0.0001**
(C) Remating latency						
	npar	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-7605.8	15220			
(1|Hemigenome line)	3	-7616.1	15238	20.57	1	**<0.0001**
(1|Replicate)	3	-7637.6	15281	63.541	1	**<0.0001**
(D) Mating rates (female biased sex ratio)						
	npar	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-192.18	392.37			
(1|Hemigenome line)	3	-192.18	390.37	0	1	1
(1|Replicate)	3	-192.2	390.41	0.037604	1	0.8462
(E) Mating rates (male biased sex ratio)						
	npar	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-208.82	425.63			
(1|Hemigenome line)	3	-209.71	425.43	1.7912	1	0.18078
(1|Replicate)	3	-210.55	427.09	3.4607	1	0.06285

Table 4.2 Outputs of linear mixed effects models for female traits showing effects of hemigenome line

Outputs of MCMCglmm Models				
		Estimate	**Lower CL**	**Upper CL**
Latency to first mating	**Female heritability**	0.0435	0.0000	0.0987
	Male heritability	0.2737	0.1003	0.4489
	Intersexual genetic correlation	-0.0176	-0.5443	0.5216
Copulation duration	**Female heritability**	0.0142	0.0000	0.0456
	Male heritability	0.1663	0.0007	0.3080
	Intersexual genetic correlation	0.2824	-0.4522	0.9772
Remating latency	**Female heritability**	0.0934	0.0287	0.1748
	Male heritability	0.2951	0.1593	0.4535
	Intersexual genetic correlation	0.0021	-0.4206	0.4543
Mating rate (male biased sex ratio)	**Female heritability**	0.2031	0.0000	0.5290
	Male heritability	0.2321	0.0000	0.5619
	Intersexual genetic correlation	0.2585	-0.5404	0.9651
Mating rate (female biased sex ratio)	**Female heritability**	0.0407	0.0000	0.1813
	Male heritability	0.1714	0.0000	0.4810
	Intersexual genetic correlation	-0.0385	-0.9403	0.8272
Fecundity of baseline females	**Heritability (female biased sex ratio)**	0.3873	0.0021	0.8184
	Heritability (male biased sex ratio)	0.0886	0.0000	0.2864
	Inter-sex ratio genetic correlation	-0.4647	-0.9960	0.2499
Sperm competitive ability	**Heritability (P1)**	0.1369	0.0000	0.2398
	Heritability (P2)	0.2458	0.0685	0.4295
	Genetic correlation between P1-P2	0.7868	0.5281	0.9975

Table 4.3 The heritabilities and additive genetic correlations for various reproduction related traits obtained using Bayesian linear mixed models

(A) Linear selection gradients on males						
Trait	**Female biased**		**Equal**		**Male biased**	
	Estimate	**SE**	**Estimate**	**SE**	**Estimate**	**SE**
Latency to first mating	**-0.1662**	**0.0458**	**-0.1897**	**0.0596**	**-0.2925**	**0.0807**
Copulation duration	0.0735	0.0519	0.1184	0.0644	0.1610	0.0901
Remating latency	**-0.1933**	**0.0428**	**-0.3137**	**0.0432**	**-0.3443**	**0.0750**
Sperm defence ability (P1)	**0.1185**	**0.0496**	**0.2978**	**0.0461**	**0.4058**	**0.0661**
Sperm offence ability (P2)	**0.1646**	**0.0460**	**0.2658**	**0.0511**	**0.4032**	**0.0666**
Mating rate	-0.0484	0.0528	-	-	0.1070	0.0923
Fecundity of baseline females	**0.1551**	**0.0465**	-	-	-0.1328	0.0913
(B) Quadratic selection gradients on males						
Trait	**Female biased**		**Equal**		**Male biased**	
	Estimate	**SE**	**Estimate**	**SE**	**Estimate**	**SE**
Latency to first mating	0.0608	0.0502	**0.1682**	**0.0604**	**0.1920**	**0.0843**
Copulation duration	-0.1191	0.0952	0.0917	0.1198	-0.0518	0.1688
Remating latency	-0.0523	0.0688	-0.0098	0.0699	0.0828	0.1206
Sperm defence ability (P1)	**-0.2457**	**0.0678**	-0.0944	0.0719	-0.1594	0.1021
Sperm offence ability (P2)	-0.0160	0.0623	-0.0042	0.0694	0.0259	0.0902
Mating rate	-0.0910	0.0965	-	-	-0.0405	0.1591
Fecundity of baseline females	-0.1181	0.0686	-	-	0.0800	0.1307

Table 4.4 Linear and quadratic selection gradients on male traits at different sex ratios. Selection gradients in bold are significantly different from 0.

(A) Linear selection gradients on females						
Trait	**Female biased**		**Equal**		**Male biased**	
	Estimate	**SE**	**Estimate**	**SE**	**Estimate**	**SE**
Latency to first mating	0.0080	0.0258	0.0086	0.0300	-0.0092	0.0384
Copulation duration	-0.0386	0.0251	**-0.0581**	**0.0284**	**-0.0773**	**0.0362**
Remating latency	0.0243	0.0255	0.0126	0.0299	0.0046	0.0384
Mating rate	-0.0043	0.0200	-	-	-0.0133	0.0358
(B) Quadratic selection gradients on females						
Trait	**Female biased**		**Equal**		**Male biased**	
	Estimate	**SE**	**Estimate**	**SE**	**Estimate**	**SE**
Latency to first mating	0.0221	0.0524	0.0202	0.0608	-0.0812	0.0768
Copulation duration	0.0009	0.0292	-0.0237	0.0329	-0.0176	0.0422
Remating latency	0.0015	0.0381	0.0346	0.0443	0.0788	0.0558
Mating rate	-0.0007	0.0272	-	-	-0.0162	0.0555

Table 4.5 Linear and quadratic selection gradients on female traits at different sex ratios. Selection gradients in bold are significantly different from 0.

(A) Pearson's product-moment correlation between female fitness and male traits						
Trait	**Female biased**		**Equal**		**Male biased**	
	Estimate	**P value**	**Estimate**	**P value**	**Estimate**	**P value**
Latency to first mating	**-0.4658**	**0.0028**	**-0.5110**	**0.0009**	-0.2615	0.1078
Copulation duration	0.2524	0.1210	0.2692	0.0975	0.3026	0.0611
Remating latency	-0.2809	0.0832	-0.3100	0.0548	-0.1722	0.2946
Sperm defence ability (P1)	**0.3622**	**0.0235**	0.3058	0.0583	0.1772	0.2805
Sperm offence ability (P2)	**0.4227**	**0.0073**	0.3140	0.0516	0.2651	0.1029
Mating rate	-0.1182	0.4861	-	-	-0.2191	0.1926
Fecundity of baseline females	0.1292	0.4461	-	-	0.0370	0.8278
(B) Pearson's product-moment correlation between male fitness and female traits						
Trait	**Female biased**		**Equal**		**Male biased**	
	Estimate	**P value**	**Estimate**	**P value**	**Estimate**	**P value**
Latency to first mating	0.1802	0.2722	0.0107	0.9485	0.0801	0.6279
Copulation duration	0.2588	0.1116	0.0949	0.5653	0.0032	0.9846
Remating latency	-0.0315	0.8490	0.0732	0.6578	0.1808	0.2706
Mating rate	**-0.3416**	**0.0385**	-	-	-0.1824	0.2800

Table 4.6 Genetic correlations between (A) female fitness and male traits, (B) male fitness and female traits

Pearson's product-moment correlation between sperm competitive ability and mating traits				
Trait	**Sperm defence ability (P1)**		**Sperm offence ability (P2)**	
	Estimate	**P value**	**Estimate**	**P value**
Latency to first mating	**-0.3424839**	**0.03799**	**-0.4766616**	**0.002858**
Copulation duration	0.2326214	0.1659	0.2510294	0.134
Remating latency	**-0.5033565**	**0.001495**	**-0.4913401**	**0.002014**
Mating rate (male biased)	0.2113224	0.2093	0.1275357	0.4519
Mating rate (female biased)	-0.1704299	0.3132	-0.2673048	0.1097

Table 4.7 Genetic correlations between sperm defence ability (P1) or sperm offence ability (P2) and male mating traits (latency to first mating, copulation duration, remating latency, mating rate at male biased sex ratio, mating rate at female biased sex ratio).

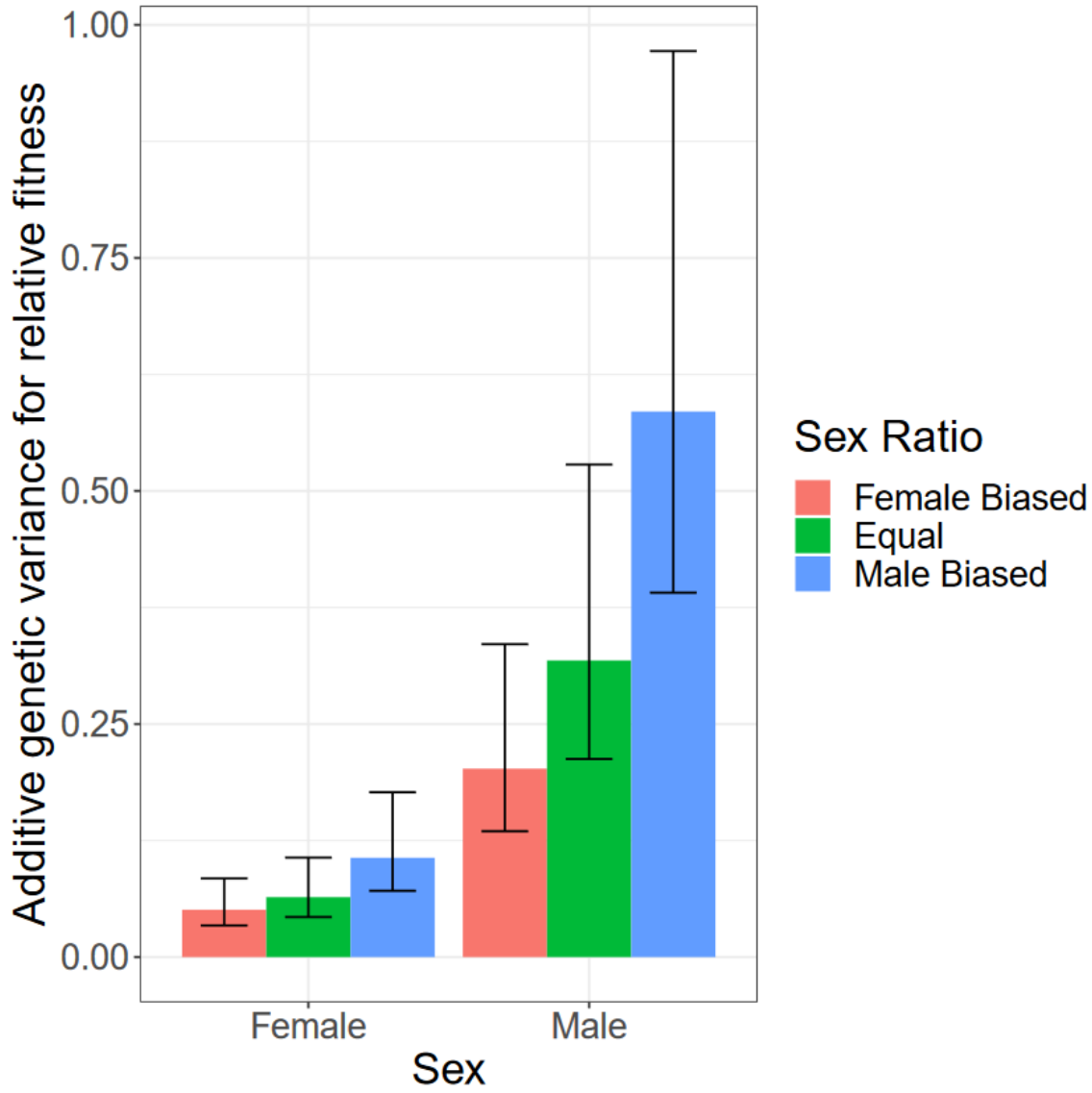

Figure 4.1 Additive genetic variance (two times the line variance) for male and female relative fitnesses at male biased, equal and female biased sex ratios (blue = male biased, green = equal, red = female biased). The errorbars indicate 95% confidence intervals generated using the χ^2 distribution.

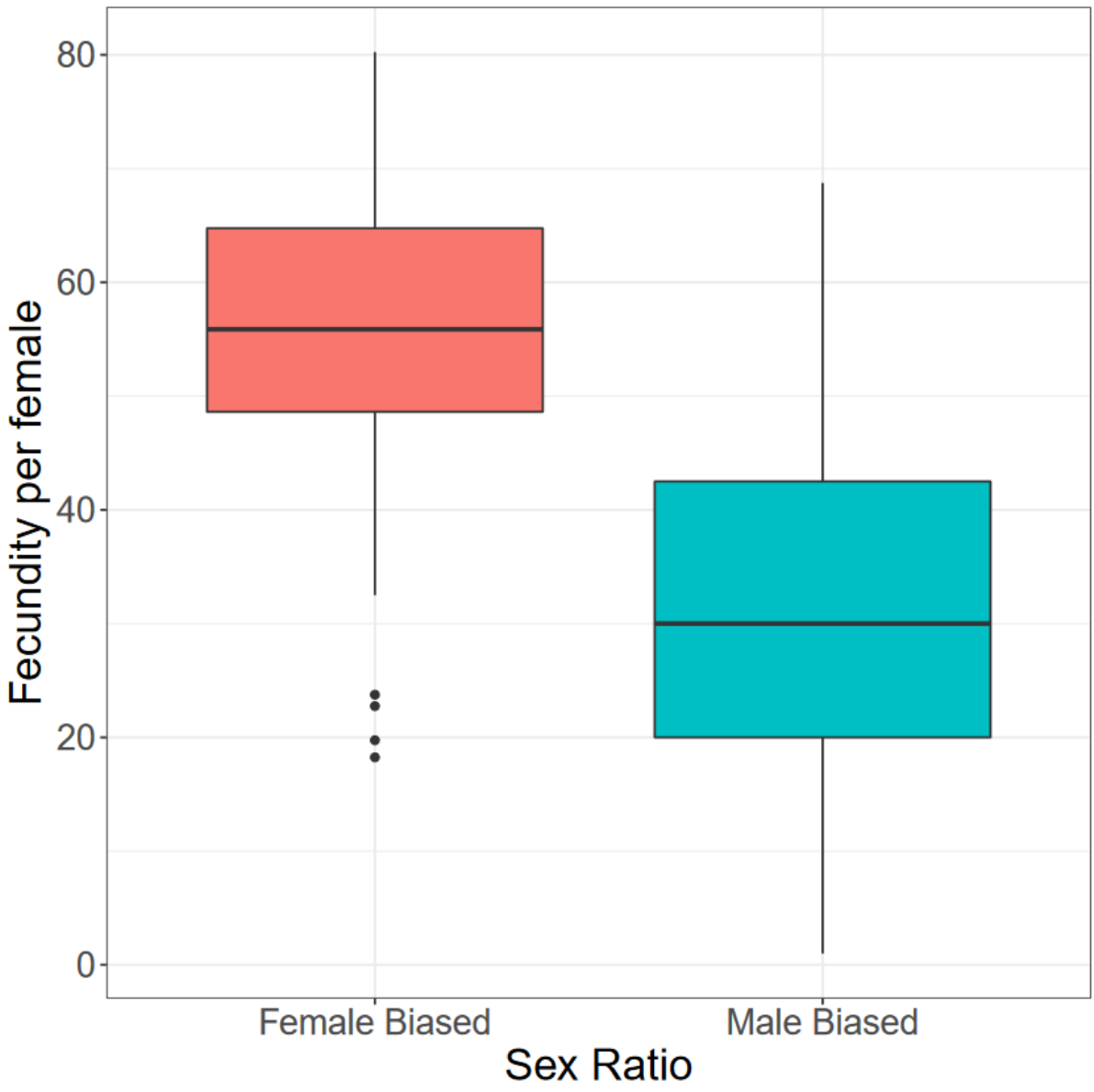

Figure 4.2 Fecundity of LHst females held with focal males at male biased and female biased sex ratios.

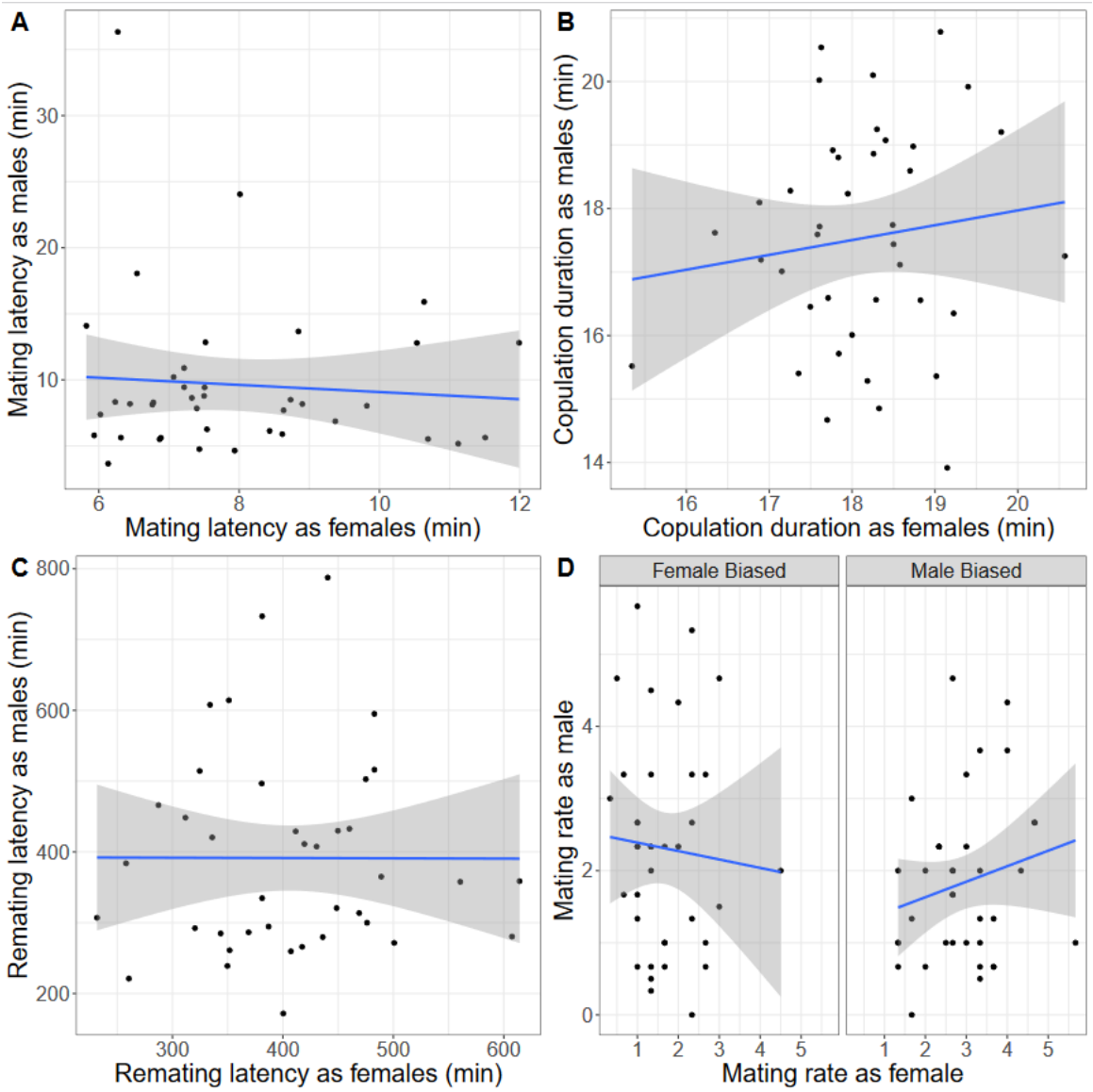

Figure 4.3 Intersexual genetic correlations for various proxies of resistance and persistence: (A) latency to first mating, (B) copulation duration, (C) remating latency, and (D) mating rates.

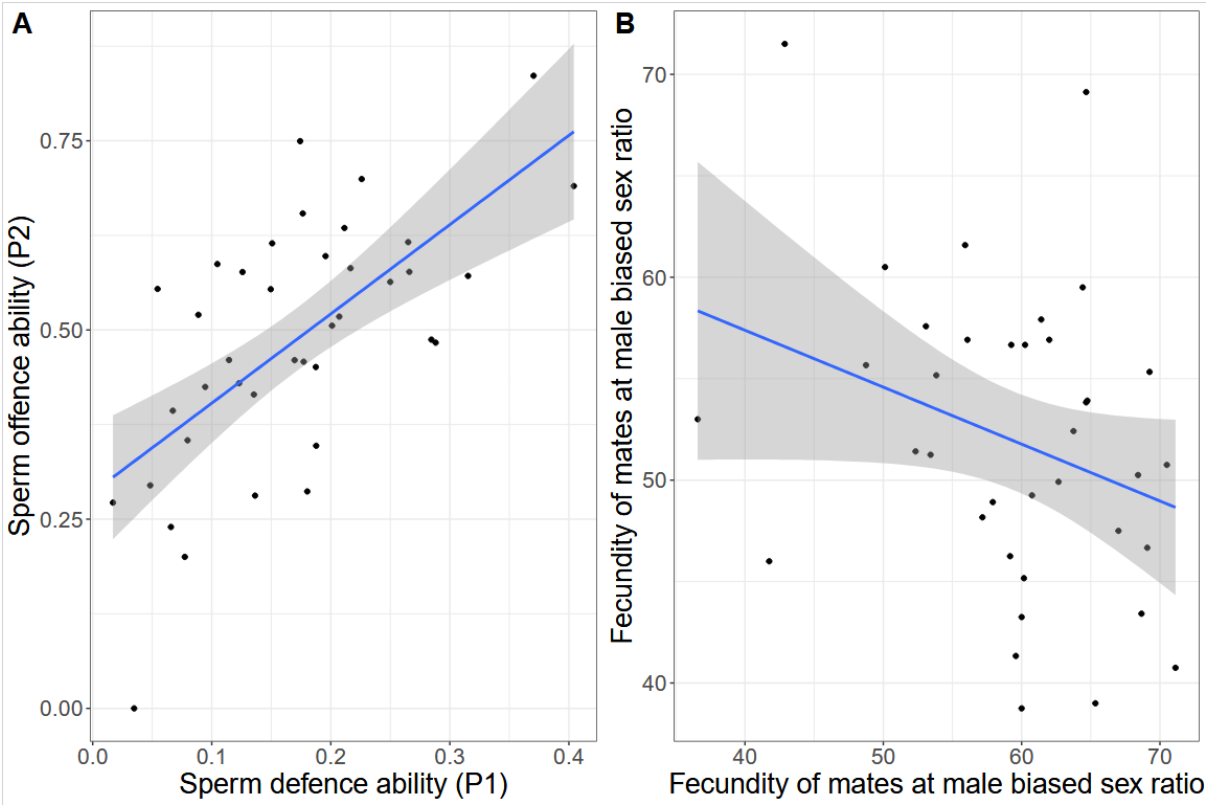

Figure 4.4 Genetic correlations (A) between sperm offence (P2) and sperm defence (P1) ability, and (B) fecundity of baseline females held with focal males at male biased or female biased sex ratios

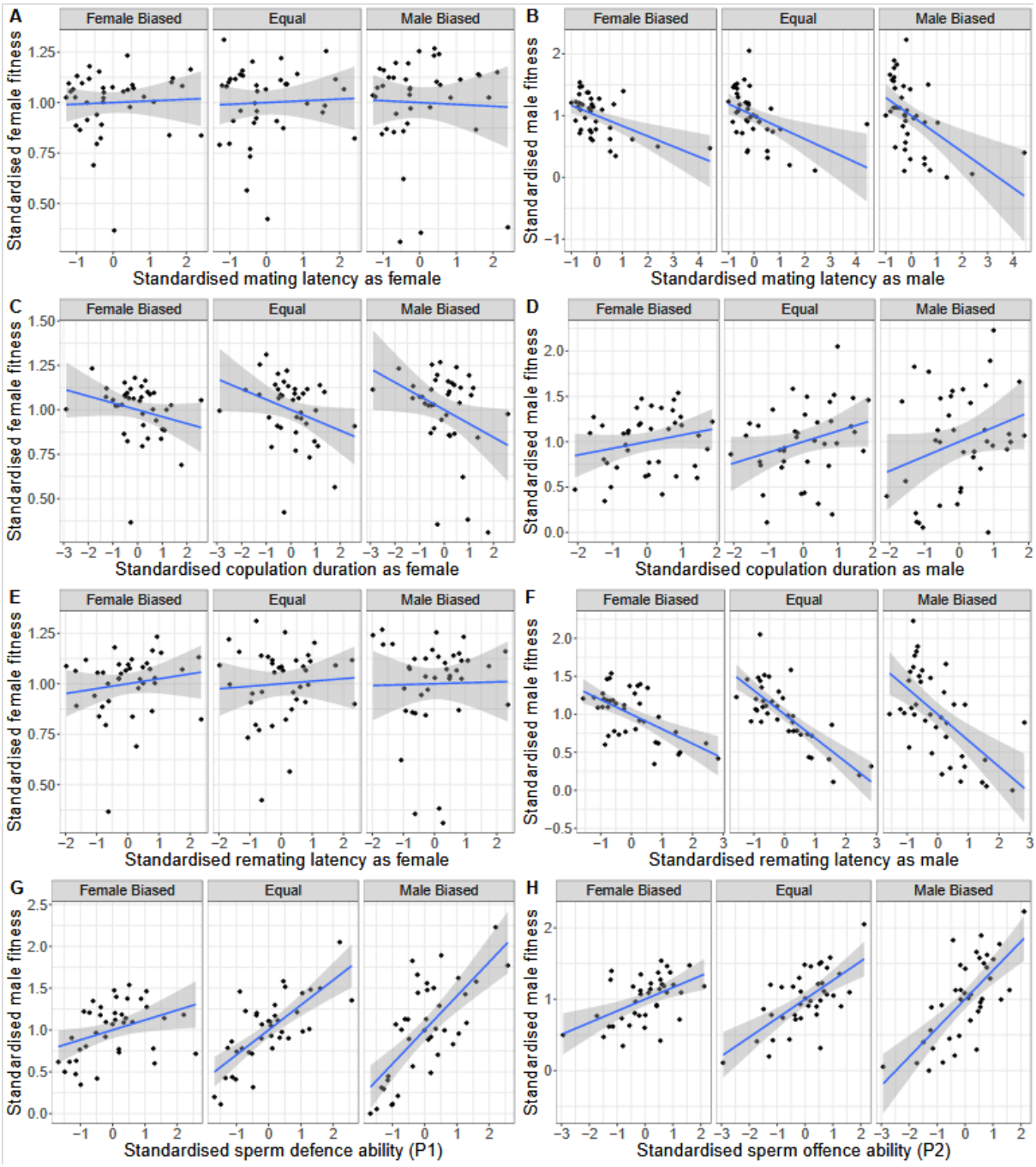

Figure 4.5 Linear selection gradients at female biased, equal and male biased sex ratios for (A) female latency to first mating, (B) male latency to first mating, (C) female copulation duration, (D) male copulation duration, (E) female remating latency, (F) male remating latency, (G) sperm defence ability (P1), and sperm offence ability (P2).

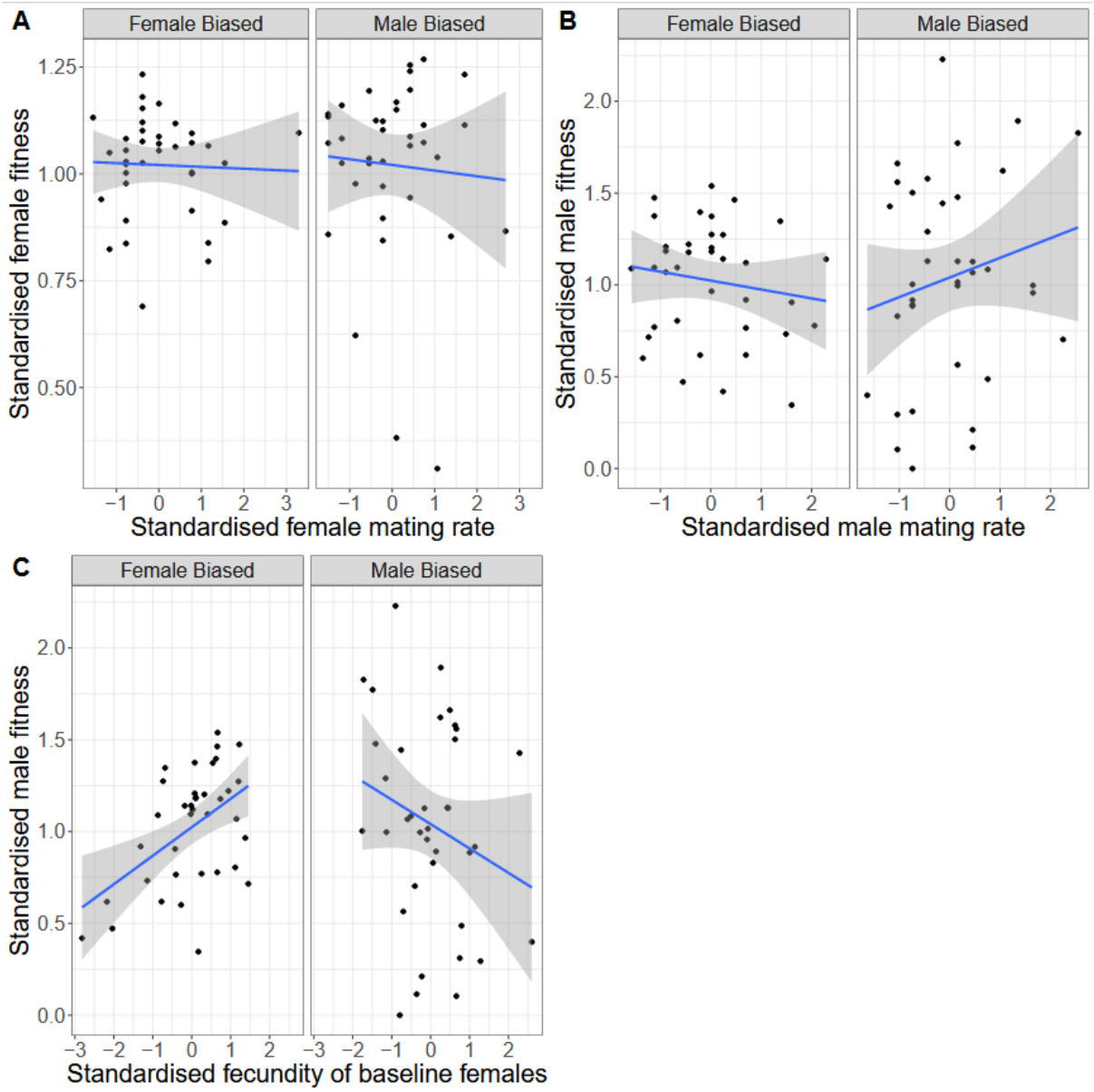

Figure 4.6 Linear selection gradients at female biased, equal and male biased sex ratios for (A) female mating rate, (B) male mating rate, (C) fecundity of baseline females held with focal males.

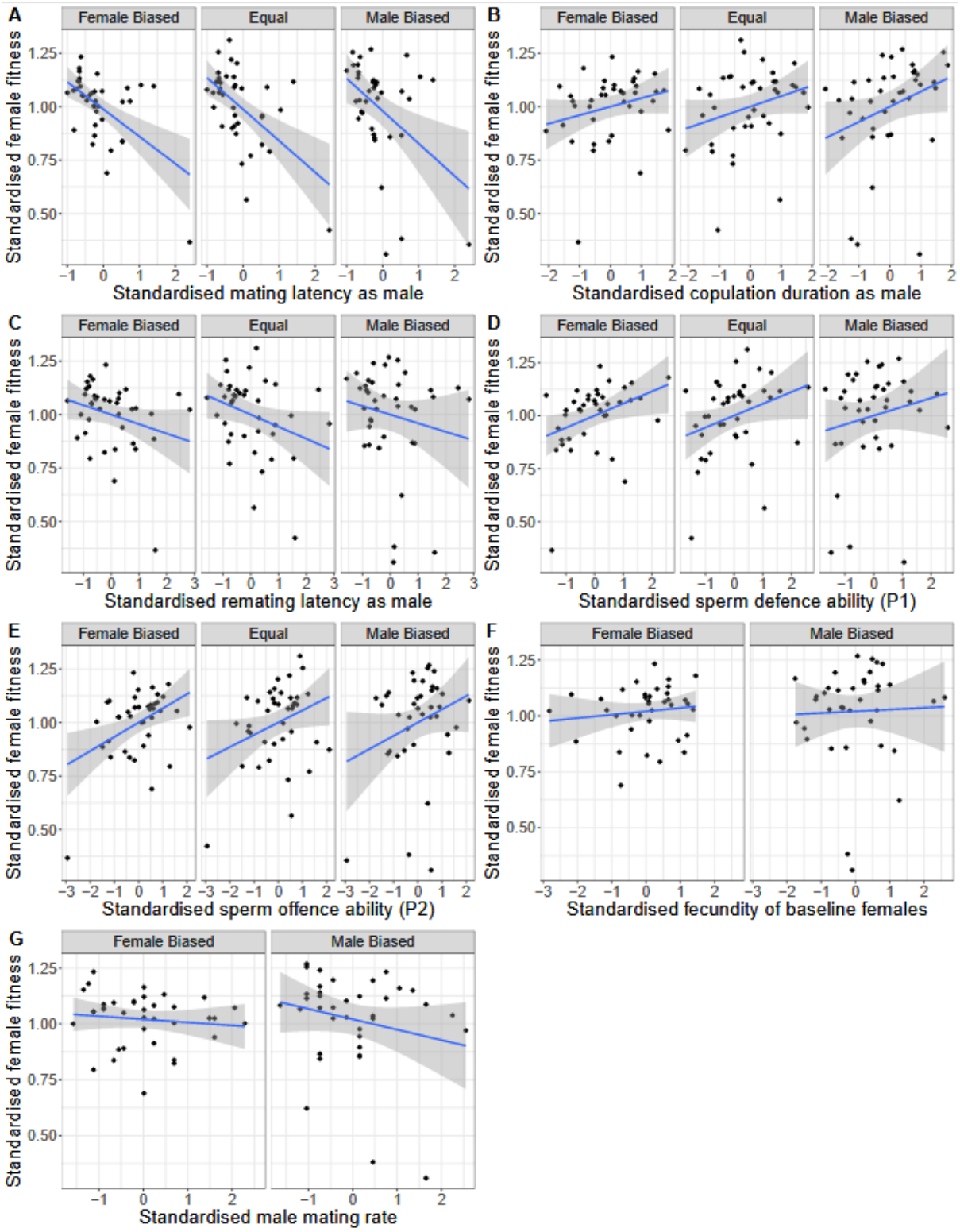

Figure 4.7 Genetic correlations of female fitness at different sex ratios with male traits: (A) latency to first mating, (B) copulation duration, (C) remating latency, (D) sperm defence ability (P1), (E) sperm offence ability (P2), (F) fecundity of baseline females held with focal males, (G) mating rate

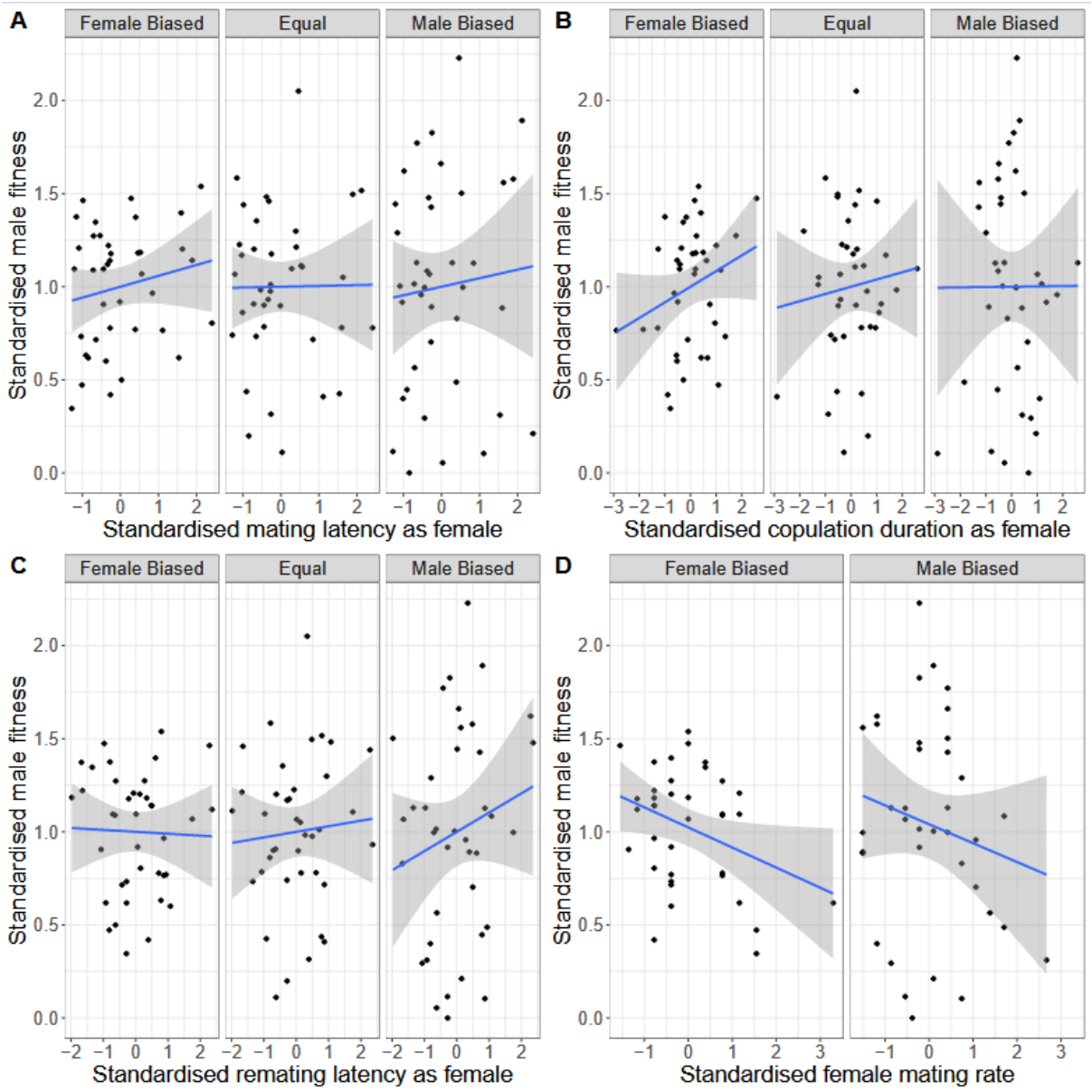

Figure 4.8 Genetic correlations of male fitness at different sex ratios with female traits: (A) latency to first mating, (B) copulation duration, (C) remating latency, (D) mating rate.

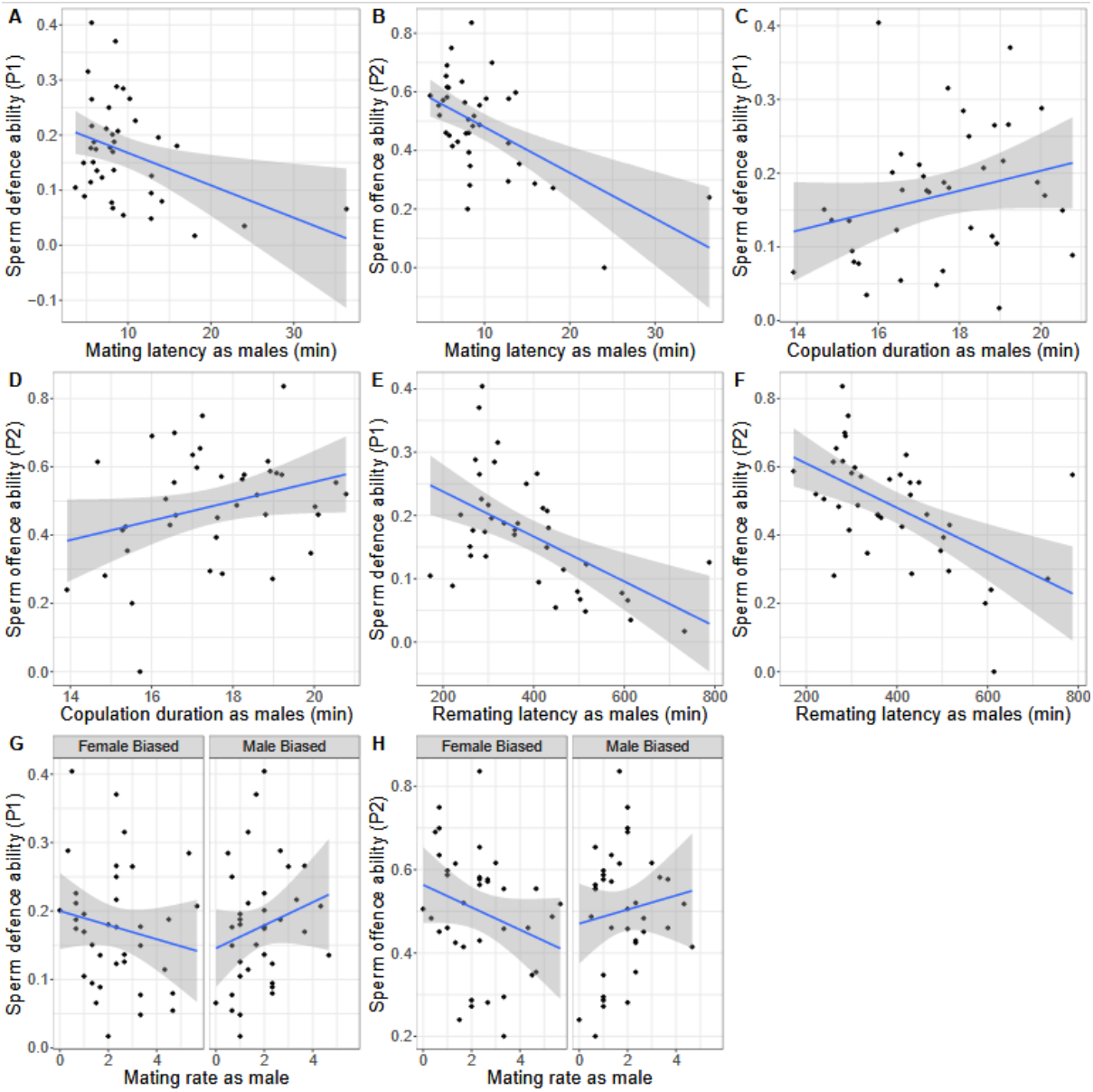

Figure 4.9 Genetic correlations between sperm defence ability (P1) and (A) latency in to first mating in males, (C) copulation duration in males, (E) Remating latency as males and (G) mating rate; as well as between sperm offence ability (P2) and (B) latency in to first mating in males, (D) copulation duration in males, (F) Remating latency as males and (H) mating rate.

Chapter 5a

Sexual dimorphism, sex-specific genetic architecture, and the nature of sex- and sex ratio-specific selection pertaining to locomotory activity, development time, and dry body weight

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INTRODUCTION

In its simplest form, natural selection is expected to lead to an increase in the frequency of the genetic variants associated with the highest Darwinian fitness, thereby pushing the population up the gradient of average population fitness, until it reaches a local optimum (Wright 1937). In more complicated scenarios, however, there can be stark differences in the evolutionary "interests" of different genetic elements, or the same genetic element in different environments, different sexes, or even different tissues, leading to situations where average population fitness need not necessarily be maximised. Examples of such genomic conflict include transposable elements, segregation distorters, mito-nuclear conflict, conflict between sex chromosomes and autosomes (Ågren and Clark 2018).

In sexually reproducing organisms, selection often favours vastly different sex-specific fitness optima, leading to sexual conflict (Schenkel et al. 2018). For example, in many species with promiscuous mating systems the optimum mating rate for males is much higher than that for females, triggering interlocus sexual conflict (IeSC). IeSC can lead to sexually antagonistic coevolution between "resistance" traits that are female-limited in their expression and "persistence" traits that are male-limited in their expression (Rowe et al. 2005). IeSC, which can be readily generalised to other aspects of reproductive interactions between males and females, has been detected in many different taxa (Daupagne and Koene 2020; Lankinen et al. 2017; McNamara et al. 2020; Sakaluk et al. 2019; Swart et al. 2020; Tomkins 2014; Wilson and Patlar et al. 2020).

In contrast to IeSC that is modelled for sex-limited traits, intra-locus sexual conflict (IaSC) occurs if the traits that have a common underlying genetic basis in the sexes (indicated by a non-zero intersexual genetic correlation) experience different sex-specific fitness optima (Bonduriansky and Chenoweth 2009). At the level of a locus, this translates to a situation

where different alleles are favoured by selection in males and females, leading to an evolutionary tug-of-war between the sexes. IaSC has the potential to prevent males and females from attaining their respective fitness optima, and keep the population at a maladaptive equilibrium. IaSC is thought to be resolved by mechanisms that bring about sex-specific genetic architecture, eventually leading to sexual dimorphism.

Patterns consistent with sexually antagonistic selection have been reported for numerous traits including locomotory activity (Long and Rice 2007), immunocompetence (Sharp and Vincent 2015; Svensson et al. 2009; Vincent and Sharp 2014;), time to reproductive maturation (Barson et al. 2015), leaf area (Delph et al. 2011), colour patterns (Price and Burley 1994). While evidence of IaSC in general is plentiful, empirical signals of IaSC can be sensitive to the environment in which they are measured. This idea stems from theoretical work by Connallon and Clark, that showed that as populations adapt to their environment, the degree of sexually antagonistic selection is expected to increase (Connallon and Clark 2014). As a corollary, the signal of IaSC should be weaker when measured in a novel environment, as opposed to an environment the population had adapted to (Connallon and Hall 2018). This hypothesis has been tested by a number of studies. Some of these studies detected an increase in sexual antagonism in novel environments (Berger et al. 2014; Long et al. 2012), while others detected no change (Holman and Jacomb 2017; Martinossi-Allibert et al. 2018) or even a reduction (Delcourt et al. 2009; Punzalan et al. 2014) in sexual antagonism in novel environments.

Most of these studies used a novel food source or a mild temperature shock as a proxy for novel environment. An important aspect of the environment that has been overlooked in these studies is the structure of the populations themselves, particularly mating systems. One of the ways in which variation in mating systems can affect the strength of IaSC in the population is through variation in sexual selection and/or IeSC in the population. Sexual selection is thought to be important for the evolution of sexual dimorphism, by promoting trait exaggeration in one of the sexes (Lande 1980). Therefore, in environments where sexual selection is stronger, the dissonance between male and female fitness optima for shared traits should be larger, thereby yielding a stronger signal of IaSC. While in their traditional formalisms, IaSC and IeSC are mutually exclusive phenomena (Fry 2010; Gavrilets et al. 2001), a number of arguments have been put forth to suggest an interaction between the two. For example, if resistance and persistence traits have negative fitness consequences when expressed in males and females respectively, strong IeSC could trigger

IaSC in the population (Pennell and Morrow 2013; Pennell et al. 2016). This implies that trait exaggeration in males should be associated with a reduction in population productivity, both as a consequence of IeSC (through male mate harm), as well as IaSC (through negative intersexual genetic correlations for fitness). Lastly, it is important to note that environments where IeSC is strong and ones where IeSC is weak or absent represent drastically different ecological scenarios. Therefore, it is likely that changes in the intensity of IeSC lead to changes in sign and/or magnitude of sex-specific selection gradients on traits that are common to males and females, thereby influencing the signal of IaSC in the population. In spite of a solid case for investigating the interaction between IaSC and IeSC, few empirical studies have sought to formally investigate such interactions.

In this study, I used hemiclonal analysis (Chippindale et al. 2001) in a laboratory adapted population of *Drosophila melanogaster* to investigate the interaction between IaSC and IeSC, by measuring how selection on three shared traits (locomotory activity, development time, and body weight) varies as one experimentally varies adult sex ratio. Varying the adult sex ratio is a common experimental technique of varying the intensity of sexual selection and IeSC (but see Kokko et al. (2012)), with intensities of sexual selection and IeSC expected to be higher at male biased sex ratios. The findings of Chapter 4 provide considerable evidence in support of this idea. For a panel of hemigenomes sampled from a laboratory adapted population of *D. melanogaster*, I measured three sexually dimorphic traits common to males and females: locomotory activity, development time, and body weight. Note that in a related population locomotory activity has been previously shown to mediate IaSC, with less active females, but more active males enjoying greater fitness (Long and Rice 2007). First, I explored the sex-specific genetic architecture for these traits. Specifically, I was interested in looking at whether there was heritable variation for any of these traits in the LH population, and whether this variation was comparable between the sexes. Additionally, to investigate if these traits could contribute to IaSC in the population, I measured intersexual genetic correlations for these traits. A significant intersexual genetic correlation would indicate that the trait is a "shared" trait between males and females, and therefore capable of modulating patterns of IaSC in the population. Using data-set of fitness at male biased, equal and female biased sex ratios described in Chapter 3, I investigated the changes in the degree of sexually antagonistic selection on three sexually dimorphic traits common to males and females: locomotory activity, development time, and body weight.

METHODS

Generating experimental flies

In this chapter, I used the panel of hemigenomes sampled in Chapter 2. I present data from 34 hemigenome lines, as 5 lines were lost in an accident. The protocol for generating experimental or focal males and females carrying the target hemigenomes in a random background from the LH population can be found in Chapter 2. Briefly, to generate experimental females and experimental males, I crossed brown eyed males from each hemigenome line (heterozygous for the translocation and target hemigenomes) with virgin LH or DxLH females respectively. I then collected red eyed female progeny from the crosses with LH females (see Figure 2.2A), and red eyed male progeny from the crosses with DxLH females (see Figure 2.2B). In the locomotory activity assay (see below), I obtained the mates for the focal flies (i.e., females in the male activity assay, and males in the female activity assay) from the LHst population. I collected eggs from the LHst population at a density of 150 eggs per vial. I performed this egg collection on the day the eggs obtained from the crosses described above were trimmed for density control, to ensure that all experimental flies were roughly of the same age at the time of the beginning of the assay assay (i.e., 2-3 old as adults).

Additionally, I used the data on male and female reproductive fitness obtained in Chapter 3 in the analyses below. I used competitive fertilization success as the measure of male fitness, and fecundity post competition for limiting amounts of live yeast as the measure of female fitness.

Locomotory activity assay

I performed separate locomotory activity assays for males and females. For the male activity assay, on the 9^{th} and 10^{th} day post egg collection, I collected focal males (red eyed males) using light CO_2 aesthesia at a density of 8 males per vial within 6 hours of eclosion to ensure that they were virgin. At the same time, I also collected LHst females as virgin at a density of 8 per vial. On the 12^{th} day post egg collection, between 6 pm and 7 pm, I combined virgin focal males from each hemigenome line with virgin LHst females in vials containing 100 microlitre supplementary live yeast paste. The concentration of the yeast paste was such that each vial had 0.47 mg supplementary yeast per female. For each hemigenome line, I set up one vial where the sex ratio was male biased (24 males: 8 females) and another where the sex ratio was female biased (8 males: 24 females). I assayed

the locomotory activity of focal males in each vial using a protocol adapted from Nandy et al (2014). I performed activity measurements at an interval of two hours, beginning at 11 PM on the 12th day post egg collection through 5 PM on the 14th day post egg collection. During each measurement, I observed a randomly chosen male from each vial continuously for two intervals of 4 seconds each. The process of random selection of males to observe was facilitated by dividing the vials into numbered sector. At the time of measurement, I generated a random number and selected a fly from the sector corresponding to that number for observation. If the sector corresponding to the number generated did not have a focal male, I generated a fresh random number. I divided vials that had 24 focal flies into 8 sectors, while those having 8 focal flies were divided into 3 sectors. After the last reading (5 PM on the 14th day post egg collection), I transferred 4 females from each vial to fresh vials. I recorded the number of eggs laid by these females over the subsequent 18 hours. The protocol for female locomotory activity was identical, except virgin focal females were combined with virgin LHst males. I performed three replicate assays for both the male and female locomotory activity assays.

Development time assay

I performed three replicate assays for male development time and three for female development time. In each replicate assay, there were two replicate vials for each hemigenome line. These vials were set up as described above (also see Chapter 2 for details). During each assay, once all flies had pupated, I counted the number of pupae in each vial. From the 9th day post egg collection, once flies started eclosing, every four hours, I flipped the flies that had eclosed into a fresh vial. These flies were frozen at -20^{o} C. Subsequently, I counted the number of focal flies of the appropriate sex (i.e., red eyed females in the female development time assay and red eyed males in the male development time assay) among them. This was done till the last fly eclosed from each vial.

Dry body weight measurements

I used the flies from the development time assay that were frozen at -20^{0} C. For each vial, I mixed the flies that had eclosed at all the different time points. I then transferred five flies from each vial to a 1.5 ml microcentrifuge tube and dried them in a hot air oven for 48 hours at 60^{o} C. I weighed these groups of five flies using a fine balance (Sartorius CPA225D) to 0.01 mg.

STATISTICAL ANALYSIS

A. Testing for the effect of sex and hemigenome line

To test for the effect of sex and hemigenome lines I fit separate linear mixed effects models for each trait (body weight, development time, locomotory activity at female biased sex ratio, and locomotory activity at male biased sex ratio) using the R packages "lme4" (Bates et al. 2022) and "lmerTest" (Kuznetsova et al. 2020). I used the following model:

Trait value ~ Sex + (1|Day) + (1|Hemigenome line) + (1|Sex:Hemigenome line)

Since in some cases adding the (1|Hemigenome line) term yielded singular fits. Therefore, I also fit the following model for each trait separately for each sex:

Trait value ~ (1|Day) + (1|Hemigenome line)

B. Genetic architecture for shared traits between males and females

In order to investigate the genetic architecture of egg to adult development time, dry body weight at eclosion and locomotory activity at male biased and female biased sex ratios, I fit Bayesian linear mixed models using the R package "MCMCglmm" (Hadfield 2010), which uses Monte-Carlo sampling techniques.

I fit two separate models to make interpretation simpler: a model including data for development time and dry body weight, and a separate model incorporating data for locomotory activity at male biased and female biased sex ratios. First, for each of the four traits (dry body weight, development time, locomotory activity at male biased sex ratio, and locomotory activity at female biased sex ratio) was standardised (mean = 0, variance = 1) separately for each replicate. Next, I fit the following model for separately for data including dry body weight and development time:

$V_{ijkmn} \sim S_i + T_j + S.T_{ij} + L_{ijk} + R.T.L_{jkm} + \varepsilon_{ijkmn}$, where V_{ijkmn} is the value of the n^{th} vial of day m, for hemigenome line k, for trait j, for sex i. S_i, T_j and $S.T_{ij}$ model the fixed effects associated with sex, trait, and the interaction between the two. L_{ijk} is a 4×4 matrix corresponding to the random effect of hemigenome line for each combination of sex and trait. L_{ijk} is modelled to follow a multivariate normal distribution with mean 0, and the variance-covariance matrix given by sex-specific additive genetic variances for each trait, additive genetic covariances between the sexes for each trait, additive genetic covariances between traits for each sex, as well as covariances between traits across sexes. $R.T.L_{jkm}$

models the random interaction between hemigenome line, trait and replicate. ε_{ijkmn} represents the matrix of sex- and trait-specific residuals, with each term modelled to follow a normal distribution with mean 0 and variance given by sex- and trait-specific residual variance. The model for locomotory activity was similar, except it did not have an index corresponding to vial, as I had only one vial corresponding to each combination of replicate, hemigenome line, sex and trait. I used these Bayesian models to calculate the estimates and 95% credible intervals (CIs) for sex-specific additive genetic (co)variances and trait-specific intersexual genetic covariances. I also specifically asked if the additive genetic variance for each of these traits was comparable between the two sexes. For each model, I ran the simulation for 100000 iterations, out of which the first 25000 were discarded as "burn-in". In the next 75000 iterations every 50^{th} iteration was sampled to create posterior distributions of the quantities of interest.

C. Signatures of sex- and sex ratio-specific selection

In order to investigate signatures of sex- and/or sex ratio-specific selection, I fit a series of linear models of increasing complexity. I calculated the relative fitness scores for each hemigenome line, for each sex and sex ratio. This was done by first calculating the averaging the fitness scores of each hemigenome line (for each sex and sex ratio) across replicate vials on the same days, and then calculating the average across days. I then calculated the relative fitness scores by dividing the average sex- and sex ratio-specific line means for fitness by the average fitness for that sex and sex ratio combination. Mean trait values for each trait for each sex were calculated using the hierarchical approach outlined above for fitness. Trait values were then standardised (mean = 0, variance = 1) separately for each sex (and sex ratio in the case of locomotory activity). I fit the following linear models:

Model 1: Relative fitness ~ Sex + Sex Ratio + Development Time + Body Weight + Locomotory Activity

Model 2: Relative fitness ~ Sex + Sex Ratio + Development Time + Body Weight + Locomotory Activity + Sex:Development Time + Sex:Body Weight + Sex:Locomotory Activity

Model 3: Relative fitness ~ Sex + Sex Ratio + Development Time + Body Weight + Locomotory Activity + Sex.Ratio:Development Time + Sex.Ratio:Body Weight + Sex Ratio: Locomotory Activity

Model 4: Relative fitness ~ Sex + Sex Ratio + Development Time + Body Weight + Locomotory Activity + Sex Ratio:Development Time + Sex.Ratio:Body Weight + Sex Ratio: Locomotory Activity + Sex:Development Time + Sex:Body Weight + Sex:Locomotory Activity

Model 5: Relative fitness ~ Sex + Sex Ratio + Development Time + Body Weight + Locomotory Activity + Sex Ratio:Development Time + Sex.Ratio:Body Weight + Sex Ratio: Locomotory Activity + Sex:Development Time + Sex:Body Weight + Sex:Locomotory Activity + Sex:Sex Ratio:Development Time + Sex:Sex Ratio:Body Weight + Sex:Sex Ratio:Locomotory Activity

Model 1 incorporates fixed effects of sex, sex ratio and the traits. Model 2, in addition to all the terms in Model 1, has interactions of all traits with sex. Model 3, in addition to all the terms in model 1, has interactions of the traits with sex ratio. Model 4 incorporates interactions of traits with both sex and sex ratio. Model 5 incorporates all the terms in model 4 and the three-way interactions between sex, sex ratio and traits. Following Holman and Jacomb (2017), I compared the AIC scores of these models to select the most appropriate model.

D. Linear and quadratic selection gradients on individual traits

Following Lande and Arnold (1983), I calculated sex- and sex ratio-specific linear and quadratic selection gradients by regressing relative fitness scores for hemigenome lines against standardised traits values and squared standardised trait values for corresponding hemigenome lines as follows. In order to calculate the linear selection gradients, I fit the following linear model separately for each combination of sex and sex ratio:

Relative fitness ~ Development Time + Body Weight + Locomotory Activity

In order to calculate quadratic and correlational selection gradients I fit the following model:

Relative fitness ~ Development Time + Body Weight + Locomotory Activity + $(\text{Development Time})^2$ + $(\text{Body Weight})^2$ + $(\text{Locomotory Activity})^2$ + (Development Time × Body Weight) + (Development Time × Locomotory Activity) + (Body Weight × Locomotory Activity)

The coefficients corresponding to the squared terms were multiplied by a factor of 2 to obtain the quadratic selection gradients (Stinchcombe et al. 2008).

E. Angle between selection in males and females

Next, for each sex ratio separately, I calculated the angle between the vectors corresponding to directional selection gradients in males and females as follows:

Angle = $cos^{-1}(\boldsymbol{\beta_m}.\boldsymbol{\beta_f}/(\sqrt{\boldsymbol{\beta_m^2}}\sqrt{\boldsymbol{\beta_f^2}})) \times 180/\pi$ where $\boldsymbol{\beta_m}$ and $\boldsymbol{\beta_f}$ are vectors corresponding to linear selection gradients in males and females.

To test if these angles were greater than expected if selection were not sex-specific, I performed a permutation test. I randomly permuted the sex of each data point to create 1000 replicate data sets. These data sets were used to construct null distributions for the angle between selection in males and females, in male biased and female biased sex ratio, if selection were not sex-specific. The actual values of angles between selection in males and females were tested against these null distributions.

To test if the angles differed between sex ratios, I randomly permuted the sex ratio of each data point, and constructed 1000 replicate datasets. These data sets were used to construct the null distribution for the difference in the angles between male biased and female biased sex ratios, against which the actual difference was tested.

F. Genetic correlation between traits and sexually antagonistic fitness variation

To investigate if the each of the traits I measured were genetically correlated with sexually antagonistic fitness variation, following Berger et al. (2014) and Ruzicka et al. (2019), I calculated the "antagonism index" (AI) for each hemigenome line for each sex ratio. First, I calculated mean sex- and sex ratio-specific fitness for each hemigenome line as described above. I standardised these mean fitness scores for each combination of sex and sex ratio (mean = 0, variance = 1). Separately for each sex ratio, I rotated the coordinate system consisting of male fitness (y-axis) and female fitness (x-axis) 45^o in the anticlockwise direction using the following operation:

$\begin{pmatrix} \bar{W}_{C,i} \\ \bar{W}_{A,i} \end{pmatrix} = \begin{pmatrix} 1/\sqrt{2} & 1/\sqrt{2} \\ -1/\sqrt{2} & 1/\sqrt{2} \end{pmatrix} \begin{pmatrix} \bar{W}_{F,i} \\ \bar{W}_{M,i} \end{pmatrix}$, where $\bar{W}_{C,i}$ and $\bar{W}_{A,i}$ are the sexually concordant fitness component and AI respectively for the hemigenome line i for that sex ratio, and $\bar{W}_{F,i}$ and $\bar{W}_{M,i}$are the average female and male fitnesses respectively for the hemigenome line i

for that sex ratio. I then regressed the sexually concordant fitness component and the AI for each sex ratio against standardised male and female trait values.

G. Correlations between SD and SA variation and fecundity of mates

In order to calculate sexual dimorphism (SD) for each trait, I first transformed sex-specific (sex- and sex ratio-specific for locomotory activity) line means to have a variance of 1, while leaving the means unchanged. I calculated SD for each hemigenome line as the difference in the transformed trait values in males and the transformed trait value in females for that trait. I also calculated the line averages for the fecundity of females housed with males from various hemigenome lines in the locomotory activity experiment. I standardised these line averages for fecundity such that they had a mean 0 and variance 1. I then regressed, separately for each sex ratio, the AI, the sexually concordant fitness component and the standardised (mean = 0, variance = 1) fecundity scores for baseline females held with focal males against SD for each trait.

RESULTS

A. Testing for the effect of sex and hemigenome line

The linear mixed model for dry body weight detected a statistically significant effect of sex, with females being heavier at eclosion than males (Figure 5a.1A), as well as well as a significant effect of hemigenome line and its interaction with sex. While males took longer than females to eclose (Figure 5a.1B), this difference was not statistically significant. Nevertheless, I detected statistically significant effects of hemigenome line, as well as its interaction with sex (Table 5a.1).

The linear mixed models locomotory activity yielded singular fits, forcing us to drop the term (1| Hemigenome line) from the models. The reduced models for both locomotory activity at male biased and female biased sex ratios yielded a statistically significant effect of sex, with males being more active than females (Figure 5a.1C). I also detected a statistically significant interaction between hemigenome line and sex for locomotory activity at both sex ratios (Table 5a.1).

When I fit separate models for males and females, for all four traits, I detected a statistically significant effect of hemigenome line, except in the case of female locomotory activity at the male biased sex ratio (Table 5a.2).

B. Additive genetic variances and covariances

I estimated the additive genetic variance-covariance matrix separately for locomotory activity at the two sex ratios, and dry body weight and development time (Table 5a.3). I found substantial additive genetic variances for both sexes for all the traits, except female locomotory activity at the male biased sex ratio. This was consistent with the linear mixed models. I also found statistically significant positive intersexual additive genetic covariances for dry body weight (0.3258, 95% CI = (0.1155, 0.6019)) and development time (0.1501, 95% CI = (0.0042, 0.3137)), but not for locomotory activity at male biased (-0.0334, 95% CI = (-0.206, 0.1030)) or female biased (-0.0748, 95% CI = (-0.2773, 0.0975)) sex ratios (Figure 5a.2).

The additive genetic covariances between dry body weight and development time were not significantly different from 0 in males (0.0262, 95% CI = (-0.1665, 0.1960)) or in females (0.06975, 95% CI = (-0.1117, 0.2516)). Similarly, the additive genetic covariance between locomotory activity at male biased and female biased sex ratios were not significantly different from 0 for females (0.0493, 95% CI = (-0.0812, 0.1979)) and males (0.2025, 95% CI = (-0.0030, 0.4233)), although the covariance in males was appreciable larger than in females.

C. Signatures of sex- and sex ratio-specific selection

The linear model for relative fitness that incorporated the interactions of the traits with sex, as well as the interactions of the traits with sex ratio (model 4) had the least AIC score (Table 5a.4).

D. Linear and quadratic selection gradients on individual traits

I investigated linear, quadratic, and correlational selection gradients on the two sexes in male biased and female biased sex ratios. These are summarised in Table 5a.3 (see Figure 5a.3 for linear selection gradients).

There was negative directional selection on dry body weight on both males and females at the female biased sex ratio, as well as on males in the male biased sex ratio. The linear selection gradient on females at the male biased sex ratio was positive, although none of the linear selection gradients on dry body weight were significantly different from 0 (Table 5a.5).

There was directional selection for early development for both males and females at both male biased and female biased sex ratios. This selection was stronger for both males and females at the male biased sex ratio, with the linear selection gradient for males at the male biased sex ratio being significantly different from 0 (Table 5a.5).

There was directional selection for more activity for males at both the sex ratios, but this trend was not statistically significant. For females, there was significantly positive directional selection at the female biased sex ratio, and negative (but statistically non-significant) selection at the male biased sex ratio.

None of the quadratic or correlational selection gradients were significantly different from 0. In males, all the quadratic selection gradients were negative, suggesting stabilising selection, except for locomotory activity at the male biased sex ratio, where there was statistically non-significant disruptive selection (Table 5a.5). By contrast, for females, all the quadratic selection gradients at the male biased sex ratio were positive, as well as the quadratic selection gradient on dry body weight at the female biased sex ratio. The quadratic selection gradients for females on development time and locomotory activity at the female biased sex ratio were positive (Table 5a.5).

E. Angle between selection in males and females

I measured the angle between the vectors of selection in males and females. A larger angle would indicate a greater dissonance in the direction of selection between the sexes, and therefore, a greater potential for IaSC. The angle between selection in males and selection in females was 27.86^{o} in the female biased sex ratio, and 47.87^{o} at male biased sex ratio. In the permutation tests, I found that these angles were not larger than expected under the null hypothesis that there was no sex-specific selection ($p = 0.815$ for female biased sex ratio, $p = 0.502$ for male biased sex ratio). Additionally, I also found that the angles between male biased and female biased sex ratios were not statistically significant ($p = 0.306$)

F. Genetic correlation between traits and sexually antagonistic or concordant fitness variation

The outputs of the linear models for AI for various traits are summarised in Table 5a.6. For both sexes, across male biased and female biased sex ratios, only female locomotory activity at female biased sex ratio was significantly associated with AI ($p = 0.039$) (Figure 5a.4). More active females were associated with negative AI, which translates to high

female fitness but low male fitness, and vice versa. Sexually concordant fitness score was significantly genetically correlated with female development time at male biased sex ratio ($p = 0.0467$), and male development time at both female biased ($p = 0.0353$) as well as male biased ($p = 0.0052$) sex ratios, with the association being stronger at the male biased sex ratios (Table 5a.7, Figure 5a.5).

G. Correlations between sexual dimorphism and AI, sexually concordant fitness component or the fecundity of baseline females held with focal males

The results of the correlations between sexual dimorphism (SD) for dry body weight, development time and locomotory activity at male biased and female biased sex ratio with AI, sexually concordant fitness component and the fecundity of baseline females are summarised in Table 5a.8.

At female biased sex ratio, only SD for locomotory activity had a positive correlation with AI (Table 5a.6A), such that more dimorphic lines were associated with greater male fitness, but poorer female fitness. At male biased sex ratio SD for development time was negatively genetically correlated with AI. The rest of the associations between AI and SD were statistically non-significant (Figure 5a.6A).

I could not detect any statistically significant genetic correlations between SD for any trait and sexually concordant fitness component at either sex ratio (Table 5a.6B, Figure 5a.6B).

None of the correlations between SD and female fecundity were statistically significant, except in the case SD for locomotory activity at female biased sex ratio, which had a positive correlation with fecundity of baseline females (Table 5a.6C, Figure 5a.6C).

DISCUSSION

Using a panel of hemigenomes sampled from a laboratory adapted population of *Drosophila melanogaster* I investigated the sex-specific genetic architecture, and the nature of sex- and sex ratio-specific selection acting on three sexually dimorphic traits: dry body weight at eclosion, development time, and adult locomotory activity. My principal findings are as follows:

(1) I found appreciable additive genetic variation in the Bayesian linear models for dry body weight, development time and adult locomotory activity at the female biased sex ratio for both sexes, but for only male locomotory activity at the male biased sex ratio. There

was little detectable additive genetic variation for female locomotory activity at the male biased sex ratio. This was also confirmed by the linear mixed models, which failed to detect an effect of hemigenome line only in the case of female locomotory activity in the male biased sex ratio.

(2) The Bayesian models detected a strong intersexual genetic correlation for development time and dry body weight, suggesting that as far as these two traits are concerned, the sexes are not free to evolve independently. However, I could not detect a statistically significant intersexual genetic correlation for locomotory activity, in either of the two sex ratios.

(3) I found some evidence to suggest that selection on the three traits under investigation was sex- and sex ratio-specific. The linear model for relative fitness that incorporated two-way interactions between the traits and sex, as well as the two-way interactions between the traits and sex ratio had the least AIC. However, when I analysed the angles between the direction of selection between the sexes in both sex ratios, I found that these angles were not higher than expected under the null hypothesis that selection was not sex specific. Additionally, the angles between the direction of selection in the sexes were not significantly different between male biased and female biased sex ratios.

(4) I could not detect any statistically significant linear selection gradients on dry body weight, at either sex ratio, or on development time at the female biased sex ratio. At the male biased sex ratio, there was strong directional selection for early eclosion for males. The linear selection gradient on female development time at the male biased sex ratio was also negative, but nor significantly different from 0. For locomotory activity, I could detect a statistically significant selection gradient only for females at the female biased sex ratio, where more active female hemigenomes were associated with greater fitness. None of the non-linear selection gradients were significantly different from 0.

(5) Female locomotory activity at the female biased sex ratio was positively genetically correlated with antagonism index (AI), while male and female development time were negatively correlated with sexually concordant fitness variation at the male biased sex ratio. Male development time was also correlated with sexually concordant fitness variation at the female biased sex ratio, but less strongly than at the male biased sex ratio.

(6) Sexual dimorphism for locomotory activity was positively genetically correlated with AI and the fecundity of baseline females held with focal males at the female biased sex

ratio. Sexual dimorphism for development time was negatively correlated with AI at the male biased sex ratio.

Below I discuss the implications of these findings, particularly in the context of the interaction between IeSC and IaSC. In Chapter 3, I had showed that experimentally increasing the intensity of IeSC led to a statistically non-significant amelioration in the signal of IaSC.

Locomotory activity is not a "shared" trait, and yet may be associated with sexual antagonism

Adult locomotory activity has been previously shown to be involved in IaSC in a population of *D. melanogaster* related to the LH population used in this study. Long and Rice (2007) showed that there was a positive intersexual genetic correlation for adult locomotory activity. At the same time, they showed that more active males, but less active females had higher fitness, leading to a conflict. In stark contrast to the findings of Long and Rice (2007), I failed to detect a statistically significant genetic correlation at either sex ratio. It could be argued that the absence of such a correlation at the male biased sex ratio is unsurprising given I failed to detect statistically significant genetic variation for female activity at male biased sex ratio. However, it is remarkable that there was no intersexual genetic correlation for locomotory activity at the female biased sex ratio as well, where I had detected appreciable additive genetic variation for locomotory activity in both sexes. My results suggest that locomotory activity is not a "shared" trait in the technical sense of the word at either of the two sex ratios. Therefore, it should no longer mediate patterns associated with IaSC in the population. An interpretation of this could be resolved IaSC over locomotory activity in the LH population. This interpretation is consistent with my findings in Chapter 3, where I reported a significantly positive intersexual genetic correlation for fitness, in contrast to previous similar studies that had reported a negative correlation, or a correlation not significantly different from 0 (Chippindale et al. 2001; Collet et al. 2016; Innocenti and Morrow 2010; Ruzicka et al. 2019). This is also consistent with a recent finding that female-limited X evolution may not lead to the evolution of adult locomotory activity, suggesting that this trait may not be under sexually antagonistic selection (Lund-Hansen et al. 2020).

My findings pertaining to the genetic correlations between male and female locomotory activity and relative fitness are also surprising in the context of previous studies. Male

locomotory activity is typically thought to be positively correlated with male fitness, primarily as more active males gain more matings (Hall 1994; Jordan et al. 2006; Partridge et al. 1987; van Dijken and Scharloo 1979). However, my results seem to suggest that, even if increased locomotory in males translates to more matings, it does not translate to increased fitness at either of the two sex ratios. This, perhaps, is a reflection of post-copulatory processes in determining male fitness. Consistent with this, Abbott et al. (2020) subjected X chromosomes to male limited evolution and could not detect any increase in male locomotory activity in the selected populations despite an increase in male reproductive fitness. Traditionally, less activity has been thought to be better for female fitness, because in *D. melanogaster*, female activity tends to stimulate male courtship (Tompkins et al. 1982) exposing females to male-induced mate harm. However, in an environment where mate harm is low (such as the female biased sex ratio environment in my experiments), this may not be the case. In such environments, females might gain a fitness advantage by being more active, especially if it gives them an edge over other females while foraging for limited nutrient-rich resources. Therefore, the positive linear selection gradient I found at the female biased sex ratio on female locomotory activity may not be entirely surprising.

One of the most intriguing aspects of my findings was that despite there being no detectable intersexual genetic correlation for locomotory activity, female locomotory activity was still associated with sexually antagonistic fitness variation in the female biased sex ratio. Hemigenomes that were more active as females (at the female biased sex ratio) have, on average, higher female fitness, but lower male fitness at the female biased sex ratio. This suggests that even when IaSC over traits per se is resolved through the evolution of sex-specific genetic architectures, loci that code for such traits may continue to have sexually antagonistic effects.

Stronger sexually concordant selection on development time at the male biased sex ratio

I found that the linear selection gradients on development time were negative for both males and females at both the sex ratios. Furthermore, these gradients were more strongly negative at the male biased sex ratio relative to the female biased sex ratio. Coupled with the fact that there was a strong positive intersexual genetic correlation for development time, these results point towards selection on development time being more sexually

concordant at the male biased sex ratio compared to the female biased sex ratio. This was confirmed by our finding that both male and female development time were strongly correlated with sexually concordant fitness variation at the male biased sex ratio. While male development time was also correlated with sexually concordant fitness variation at the female biased sex ratio, this relationship was much weaker than at the male biased sex ratio.

It is intriguing that there was strong selection for early development in my experiments at the male biased sex ratio, particularly in the context of studies that explicitly selected populations for early development and reported reduced reproductive output (Chippindale et al. 1997; Joshi et al. 2001; Nunney 1996; Sharma and Shakarad 2021; but see Zwaan et al. 1995). Nevertheless, it may not be prudent to compare my results with studies employing strong selection for early development, as these studies were able to reduce the development time of the selected populations much beyond the variation in development time in the ancestral baseline populations. For example, Prasad et al. (2000), who used the populations described by Joshi et al. (2001), selected for early development and early reproduction and reported that after about 70 generations of selection, the difference between selected and control populations for egg to adult had grown to around 36 hours. Similarly, Chippindale et al. (1997) observed a reduction in the development time of their selected populations by more than 30 hours. This is sharp contrast to my study, where my analyses were restricted to the standing genetic variation for development time.

My results are also important in the context of studies employing sex-limited evolution to detect the presence of sexually antagonistic fitness variation. Prasad et al. (2007) exposed entire genomes of *D. melanogaster* to male-limited evolution and reported that male fitness increased, but female fitness decreased in the male-limited populations relative to controls. They also found that flies from the male-limited populations were slower to develop, suggesting that development time was associated with sexually antagonistic fitness variation, such that slower development was better for male fitness but not for female fitness. On the other hand, Lund-Hansen et al. (2020) subjected *D. melanogaster* X chromosomes to female-limited evolution and detected a decrease in the development time of the selected populations relative to the controls, which was not accompanied by any change in reproductive fitness.

One of the interpretations of my results could be the accumulation of some sexually concordant deleterious mutations either in the LH population itself, or in some of my hemigenome lines. It is possible that such mutations make flies (male or female) less fit in every sense, i.e., they develop slowly, are less fecund as females and are less competitive as males.

No evidence that greater sexual dimorphism is associated with increased mate harm

Pennell and Morrow (2013) had argued that processes that resolve IaSC, could trigger IeSC as trait exaggeration in males driving sexual dimorphism could translate to increased mate harm towards females. In my experiments this would have translated to a negative correlation between sexual dimorphism and the fecundity of baseline females held with focal males at the male biased sex ratio. However, I found no evidence in favour of this idea. At the male biased sex ratio, I detected no correlations between sexual dimorphism and the fecundity of baseline females, suggesting that more sexually dimorphic hemigenomes were not necessarily more harmful towards females. On the other hand, at the female biased sex ratio, there was a positive correlation between sexual dimorphism for locomotory activity and fecundity of baseline females. These results are in stark contrast to the findings of (Berger et al. 2016) who had reported reduced line productivities in isofemale lines of *C. maculates* exhibiting greater sexual dimorphism for multivariate traits linked to life-history as well as morphology.

CONCLUSION

Selection on development time. Dry body weight and locomotory activity was sex- and sex ratio-specific. My findings suggest that locomotory activity is not a shared trait between males and females in the LH population. Therefore, it cannot mediate patterns of IaSC in the population. However, female locomotory activity at female biased sex ratio was significantly genetically correlated with sexually antagonistic fitness variation. I found strong sexually concordant selection on development time at male biased sex ratio. This could potentially explain the results of Chapter 3, where I found an amelioration in the signal of IaSC at male biased sex ratio.

Outputs of mixed model ANOVAs Satterthwaite's method to test for the effect of sex						
(A) Effect of sex						
Trait	**Sum Sq**	**Mean Sq**	**NumDF**	**DenDF**	**F value**	**Pr(>F)**
Body weight	0.0218	0.0218	1	4.7658	67.9440	**0.0005**
Development time	2.6445	2.6445	1	4.0582	0.2742	0.6278
Activity (female biased)	0.4112	0.4112	1	18.0260	36.5700	**<0.0001**
Activity (male biased)	0.5069	0.5069	1	15.2800	51.7360	**<0.0001**
(B) Random effects						
Body Weight	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	6	950.42	-1888.8			
(1|Day)	5	917.62	-1825.2	65.606	1	**<0.0001**
(1|Family)	5	942.59	-1875.2	15.659	1	**<0.0001**
(1|Family:Sex)	5	937.26	-1864.5	26.315	1	**<0.0001**
Development time	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	6	-1103.3	2218.7			
(1|Day)	5	-1400.2	2810.3	593.65	1	**<0.0001**
(1|Family)	5	-1114.5	2239	22.33	1	**<0.0001**
(1|Family:Sex)	5	-1125.4	2260.8	44.11	1	**<0.0001**
Activity (female biased sex ratio)	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	6	141.36	-270.72			
(1|Day)	5	141.35	-272.7	0.0196	1	0.8886
(1|Family:Sex)	5	131.74	-253.49	19.2355	1	**<0.0001**
Activity (male biased sex ratio)	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	6	159.35	-306.7			
(1|Day)	5	159.34	-308.67	0.0303	1	0.8618
(1|Family:Sex)	5	152	-294.01	14.6951	1	**0.0001**

Table 5a.1 The outputs of linear mixed effects models for development time, dry body weight, locomotory activity at female biased and male biased sex ratios.

Outputs of mixed model ANOVAs Satterthwaite's method to test for the effect of hemigenome line						
(A) Females						
Body Weight	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	461.13	-914.25			
(1|Day)	3	440.8	-875.6	4.07E+01	1	**<0.0001**
(1|Family)	3	428.46	-850.92	6.53E+01	1	**<0.0001**
Development time	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-559.28	1126.6			
(1|Day)	3	-660.74	1327.5	202.9	1	**<0.0001**
(1|Family)	3	-620.38	1246.8	122.2	1	**<0.0001**
Activity (female biased sex ratio)	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	105.82	-203.65			
(1|Day)	3	104.65	-203.31	2.337	1	0.1263
(1|Family)	3	101.6	-197.2	8.4498	1	**0.0037**
Activity (male biased sex ratio)	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	99.759	-191.52			
(1|Day)	3	99.497	-192.99	0.52349	1	0.4694
(1|Family)	3	98.733	-191.47	2.0516	1	0.152
(B) Males						
Body Weight	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	487.94	-967.88			
(1|Day)	3	478.35	-950.7	19.178	1	**<0.0001**
(1|Family)	3	429.84	-853.69	116.191	1	**<0.0001**
Development time	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	-550.61	1109.2			
(1|Day)	3	-727.56	1461.1	353.89	1	**<0.0001**
(1|Family)	3	-630.86	1267.7	160.49	1	**<0.0001**
Activity (female biased sex ratio)	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	48.89	-89.779			
(1|Family)	3	42.104	-78.208	13.571	1	**0.0002**
Activity (male biased sex ratio)	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	4	64.69	-121.38			
(1|Family)	3	56.957	-107.91	15.466	1	**0.0001**

Table 5a.2 The outputs of linear mixed effects models for development time, dry body weight, locomotory activity at female biased and male biased sex ratios to test for hemigenome line.

(A) Additive genetic (co)variance matrix for dry body weight and development time					
		Female		**Male**	
		Dry body weight	**Development time**	**Dry body weight**	**Development time**
Female	**Dry body weight**	0.3000 (0.0716, 0.5999)	0.06975 (-0.1117, 0.2516)	**0.3258 (0.1155, 0.6019)**	0.0251 (-0.1281, 0.1713)
	Development time	0.06975 (-0.1117, 0.2516)	0.2537 (0.0264, 0.4970)	0.0953 (-0.1036, 0.3049)	**0.1501 (0.0042, 0.3137)**
Male	**Dry body weight**	**0.3258 (0.1155, 0.6019)**	0.0953 (-0.1036, 0.3049)	0.5153 (0.1904, 0.9605)	0.0262 (-0.1665, 0.1960)
	Development time	0.0251 (-0.1281, 0.1713)	**0.1501 (0.0042, 0.3137)**	0.0262 (-0.1665, 0.1960)	0.1445 (0.0002, 0.3166)
(B) Additive genetic (co)variance matrix for locomotory activity					
		Female		**Male**	
		Activity (female biased)	**Activity (male biased)**	**Activity (female biased)**	**Activity (male biased)**
Female	**Activity (female biased)**	0.2914 (<0.0001, 0.5660)	0.0493 (-0.0812, 0.1979)	-0.0748 (-0.2773, 0.0975)	-0.0239 (-0.2223 0.1811)
	Activity (male biased)	0.0493 (-0.0812, 0.1979)	0.0968 (<0.0001, 0.2972)	-0.0106 (-0.1521, 0.1266)	-0.0334 (-0.206, 0.1030)
Male	**Activity (female biased)**	-0.0748 (-0.2773, 0.0975)	-0.0106 (-0.1521, 0.1266)	0.3031(0.0088, 0.6161)	0.2025 (-0.0030, 0.4233)
	Activity (male biased)	-0.0239 (-0.2223 0.1811)	-0.0334 (-0.206, 0.1030)	0.2025 (-0.0030, 0.4233)	0.4568 (0.1493, 0.7816)

Table 5a.3. The additive genetic (co)variance matrix for (A) dry body weight and development time, (B) locomotory activity. Figures in parentheses indicate 95% credible intervals.

Model	Formula	AIC
Model 1	Relative fitness ~ Sex + Sex Ratio + Development Time + Body Weight + Locomotory Activity	73.0669
Model 2	Relative fitness ~ Sex + Sex Ratio + Development Time + Body Weight + Locomotory Activity + Sex:Development Time + Sex:Body Weight + Sex:Locomotory Activity	68.2532
Model 3	Relative fitness ~ Sex + Sex Ratio + Development Time + Body Weight + Locomotory Activity + Sex.Ratio:Development Time + Sex.Ratio:Body Weight + Sex Ratio: Locomotory Activity	72.2556
Model 4	**Relative fitness ~ Sex + Sex Ratio + Development Time + Body Weight + Locomotory Activity + Sex Ratio:Development Time + Sex.Ratio:Body Weight + Sex Ratio: Locomotory Activity + Sex:Development Time + Sex:Body Weight + Sex:Locomotory Activity**	**67.1136**
Model 5	Relative fitness ~ Sex + Sex Ratio + Development Time + Body Weight + Locomotory Activity + Sex Ratio:Development Time + Sex.Ratio:Body Weight + Sex Ratio: Locomotory Activity + Sex:Development Time + Sex:Body Weight + Sex:Locomotory Activity + Sex:Sex Ratio:Development Time + Sex:Sex Ratio:Body Weight + Sex:Sex Ratio:Locomotory Activity	70.0014

Table 5a.4 The AIC scores for linear models aimed at investigating sex- and sex ratio-specific selection on dry body weight, development time, and locomotory activity.

(A) Linear selection gradients					
		Females		**Males**	
		Estimate	**Standard error**	**Estimate**	**Standard error**
Female biased sex ratio	Dry body weight	-0.0034	0.0198	-0.0219	0.0482
	Development time	-0.0113	0.0195	-0.0640	0.0535
	Locomotory activity	**0.0480**	**0.0198**	0.0760	0.0541
Male biased sex ratio	Dry body weight	0.0300	0.0373	-0.0665	0.0852
	Development time	-0.0761	0.0390	**-0.2772**	**0.0845**
	Locomotory activity	-0.0022	0.0391	0.0835	0.0867
(B) Quadratic selection gradients					
		Females		**Males**	
		Estimate	**Standard error**	**Estimate**	**Standard error**
Female biased sex ratio	Dry body weight	0.0393	0.0401	-0.1196	0.0675
	Development time	-0.0089	0.0439	-0.0978	0.1179
	Locomotory activity	-0.0213	0.0487	-0.0296	0.0830
Male biased sex ratio	Dry body weight	0.0817	0.0744	-0.0600	0.1646
	Development time	0.0417	0.0820	-0.1335	0.1956
	Locomotory activity	0.0278	0.0631	0.1174	0.1277
(C) Correlational selection gradients					
		Females		**Males**	
		Estimate	**Standard error**	**Estimate**	**Standard error**
Female biased sex ratio	Dry body weight - Development time	0.0297	0.0262	0.0513	0.0609
	Dry body weight - Locomotory activity	-0.0297	0.0225	0.0886	0.0864
	Locomotory activity - Development time	0.0011	0.0209	0.0900	0.0910
Male biased sex ratio	Dry body weight - Development time	-0.0411	0.0550	-0.1112	0.1120
	Dry body weight - Locomotory activity	-0.0988	0.0613	0.0433	0.1770
	Locomotory activity - Development time	0.0766	0.0675	-0.0025	0.1322

Table 5a.5 Summary of (A) linear, (B) quadratic and (C) correlational selection gradients on dry body weight, development time and locomotory activity at male biased and female biased sex ratio. Values in bold indicate selection gradients significantly different from 0.

Linear models for antagonism index (AI) ~ trait					
(A) Female biased sex ratio					
	Trait	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Females	Dry body weight	-0.2399	0.1664	-1.4420	0.1590
	Development time	-0.1458	0.1698	-0.8590	0.3970
	Locomotory activity	**-0.3454**	**0.1605**	**-2.1520**	**0.0390**
Males	Dry body weight	-0.1122	0.1706	-0.6580	0.5150
	Development time	-0.1119	0.1706	-0.6560	0.5170
	Locomotory activity	0.2133	0.1675	1.2730	0.2120
(B) Male biased sex ratio					
	Trait	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Females	Dry body weight	-0.2300	0.1417	-1.6230	0.1140
	Development time	0.0827	0.1467	0.5640	0.5770
	Locomotory activity	-0.1165	0.1460	-0.7980	0.4310
Males	Dry body weight	-0.0215	0.1474	-0.1460	0.8850
	Development time	-0.1611	0.1447	-1.1130	0.2740
	Locomotory activity	0.1038	0.1463	0.7100	0.4830

Table 5a.6. Summary of linear models for antagonism indices for male and female dry body weight, development time, and locomotory activity at (A) female biased sex ratio and (B) male biased sex ratio.

Linear models for sexually concordant fitness variation ~ trait					
(A) Female biased sex ratio					
	Trait	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Females	Dry body weight	-0.1710	0.1792	-0.9540	0.3470
	Development time	-0.2260	0.1773	-1.2750	0.2120
	Locomotory activity	0.2171	0.1776	1.2230	0.2300
Males	Dry body weight	-0.0521	0.1815	-0.2870	0.7760
	Development time	**-0.3723**	**0.1694**	**-2.1980**	**0.0353**
	Locomotory activity	0.3174	0.1728	1.8360	0.0756
(B) Male biased sex ratio					
	Trait	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Females	Dry body weight	-0.0954	0.2012	-0.4740	0.6390
	Development time	**-0.3924**	**0.1896**	**-2.0690**	**0.0467**
	Locomotory activity	0.0622	0.2016	0.3090	0.7600
Males	Dry body weight	-0.1128	0.2009	-0.5620	0.5780
	Development time	**-0.5351**	**0.1784**	**-3.0000**	**0.0052**
	Locomotory activity	-0.0562	0.2016	-0.2790	0.7820

Table 5a.7. Summary of linear models for sexually concordant fitness scores for male and female dry body weight, development time, and locomotory activity at (A) female biased sex ratio and (B) male biased sex ratio.

Linear models with sexual dimorphism as explanatory variable					
(A) Antagonism index and sexual dimorphism					
	Trait	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Females biased sex ratio	Dry body weight	0.1613	0.1909	0.8450	0.4040
	Development time	0.0542	0.2168	0.2500	0.8040
	Locomotory activity	**0.2547**	**0.1068**	**2.3840**	**0.0230**
Males biased sex ratio	Dry body weight	0.26347	0.1591	1.6560	0.1070
	Development time	**-0.3894**	**0.1732**	**-2.2490**	**0.0316**
	Locomotory activity	0.0971	0.0964	1.0070	0.3210
(B) Sexually concordant fitness component and sexual dimorphism					
	Trait	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Females biased sex ratio	Dry body weight	0.1502	0.2025	0.7420	0.4640
	Development time	-0.2338	0.2259	-1.0350	0.3080
	Locomotory activity	0.0457	0.1224	0.3730	0.7110
Males biased sex ratio	Dry body weight	-0.0220	0.2269	-0.0970	0.9230
	Development time	-0.2281	0.2520	-0.9050	0.3720
	Locomotory activity	-0.0522	0.1337	-0.3900	0.6990
(C) Fecundity of baseline females and sexual dimorphism					
	Trait	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Females biased sex ratio	Dry body weight	0.0917	0.1980	0.4630	0.6470
	Development time	-0.3354	0.2154	-1.5570	0.1290
	Locomotory activity	**0.2405**	**0.1115**	**2.1570**	**0.0387**
Males biased sex ratio	Dry body weight	-0.0249	0.1987	-0.1260	0.9010
	Development time	0.01234	0.2234	0.0550	0.9560
	Locomotory activity	-0.1608	0.1139	-1.4120	0.1670

Table 5a.8. Summary of linear models with (A) antagonism index, (B) sexually concordant fitness component, or (C) fecundity of baseline females held with focal males as the response variable, and sexual dimorphism for the three traits as the independent variable.

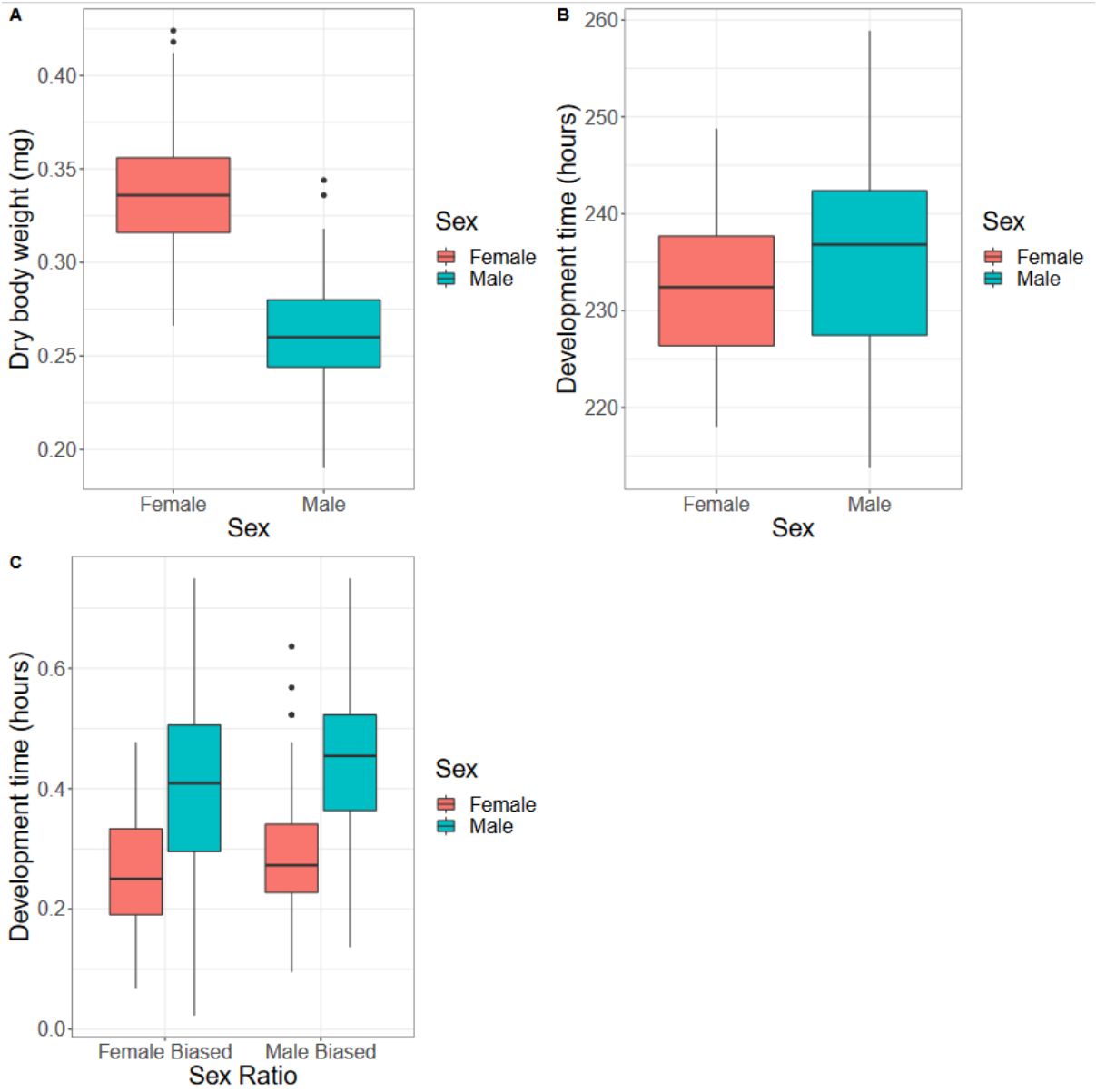

Figure 5a.1 Sexual dimorphism for (A) dry body weight, (B) egg to adult development time, and (C) adult locomotory activity at male biased and female biased sex ratios.

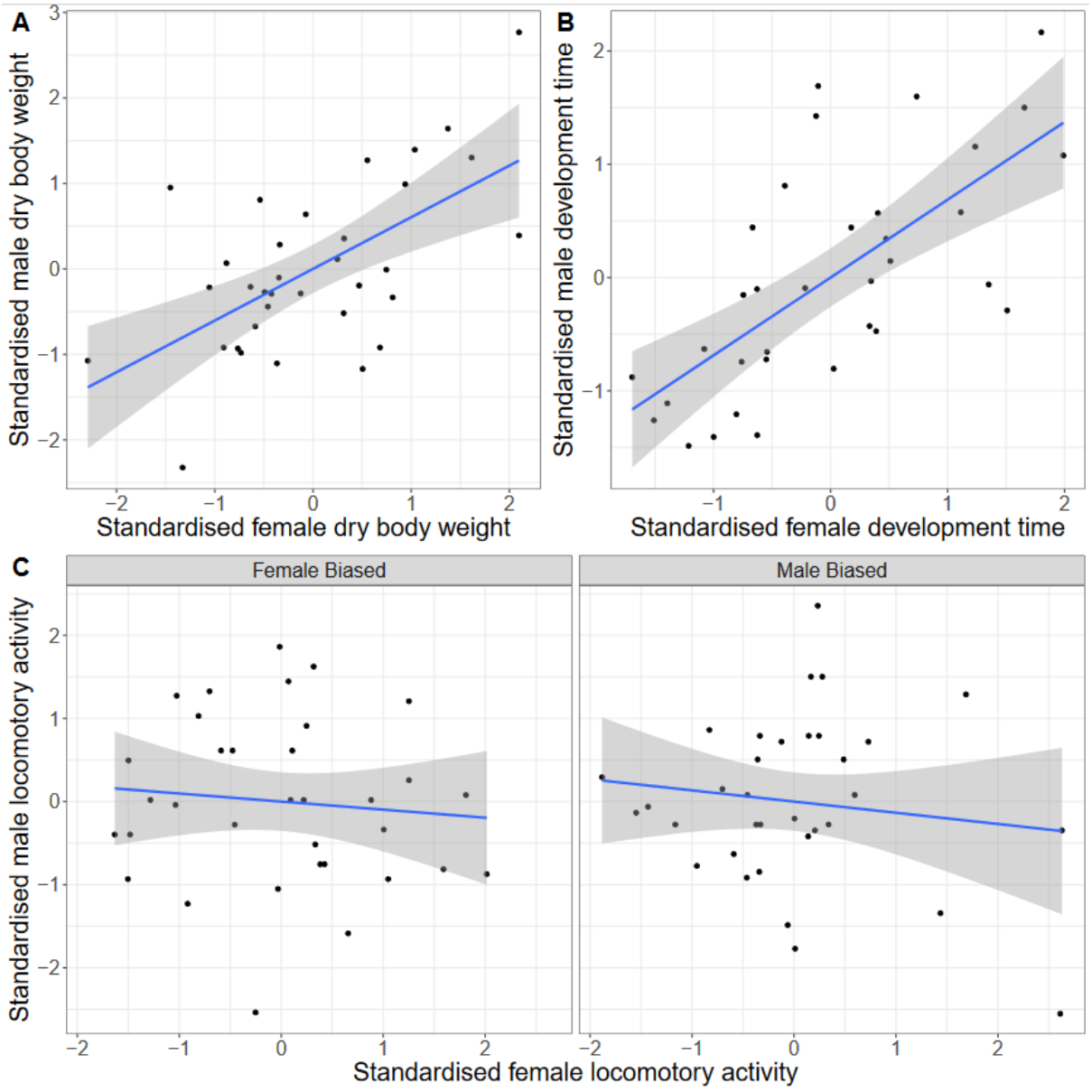

Figure 5a.2 Intersexual genetic correlations for (A) dry body weight, (B) egg to adult development time, and (C) adult locomotory activity.

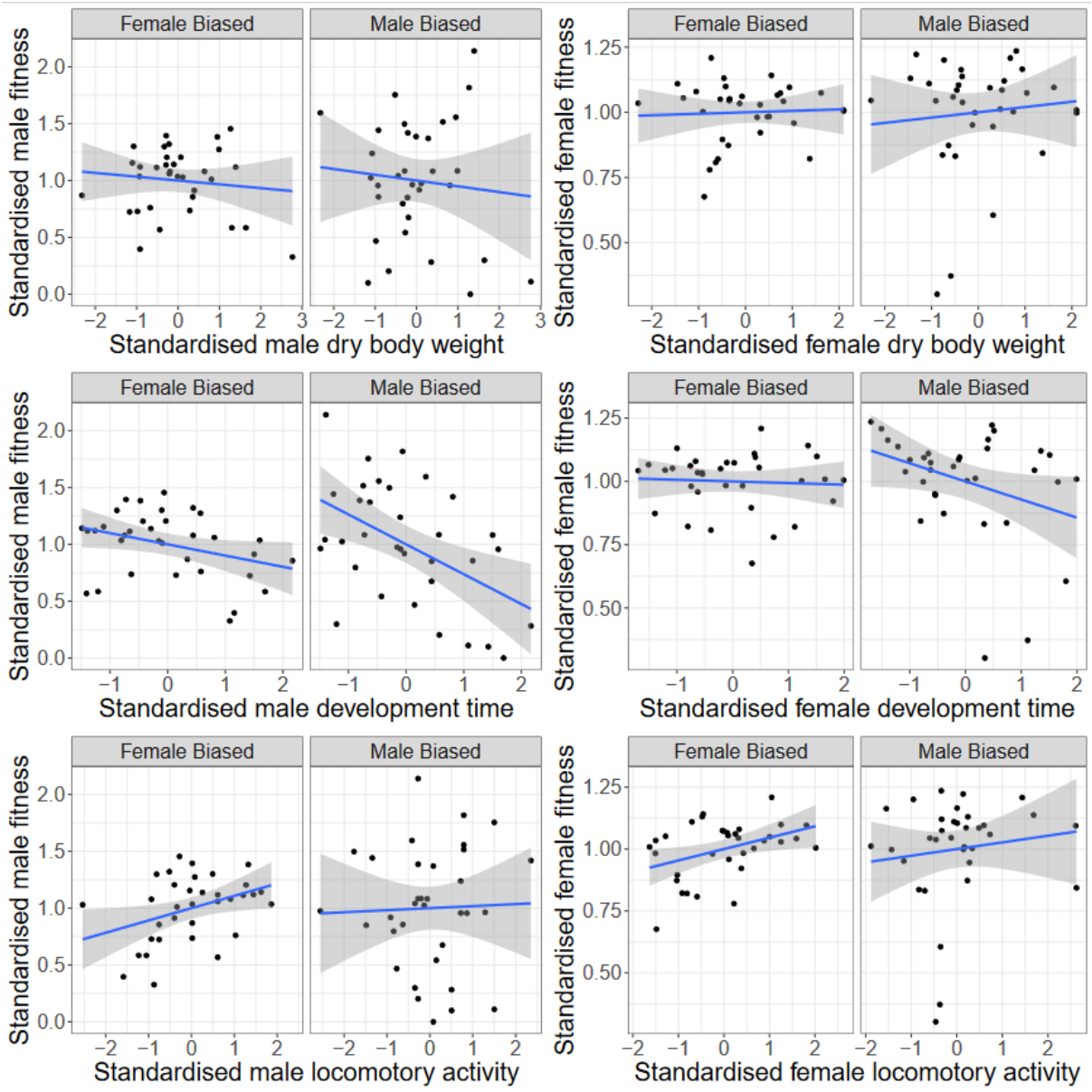

Figure 5a.3 Genetic correlations between relative male and female fitness at male biased or female biased sex ratio and dry body weight, egg to adult development time and adult locomotory activity. The column on the left represents correlations for males, while the columns on the right represents correlations for females. First, second and third rows represent dry body weight, development time, and locomotory activity, respectively.

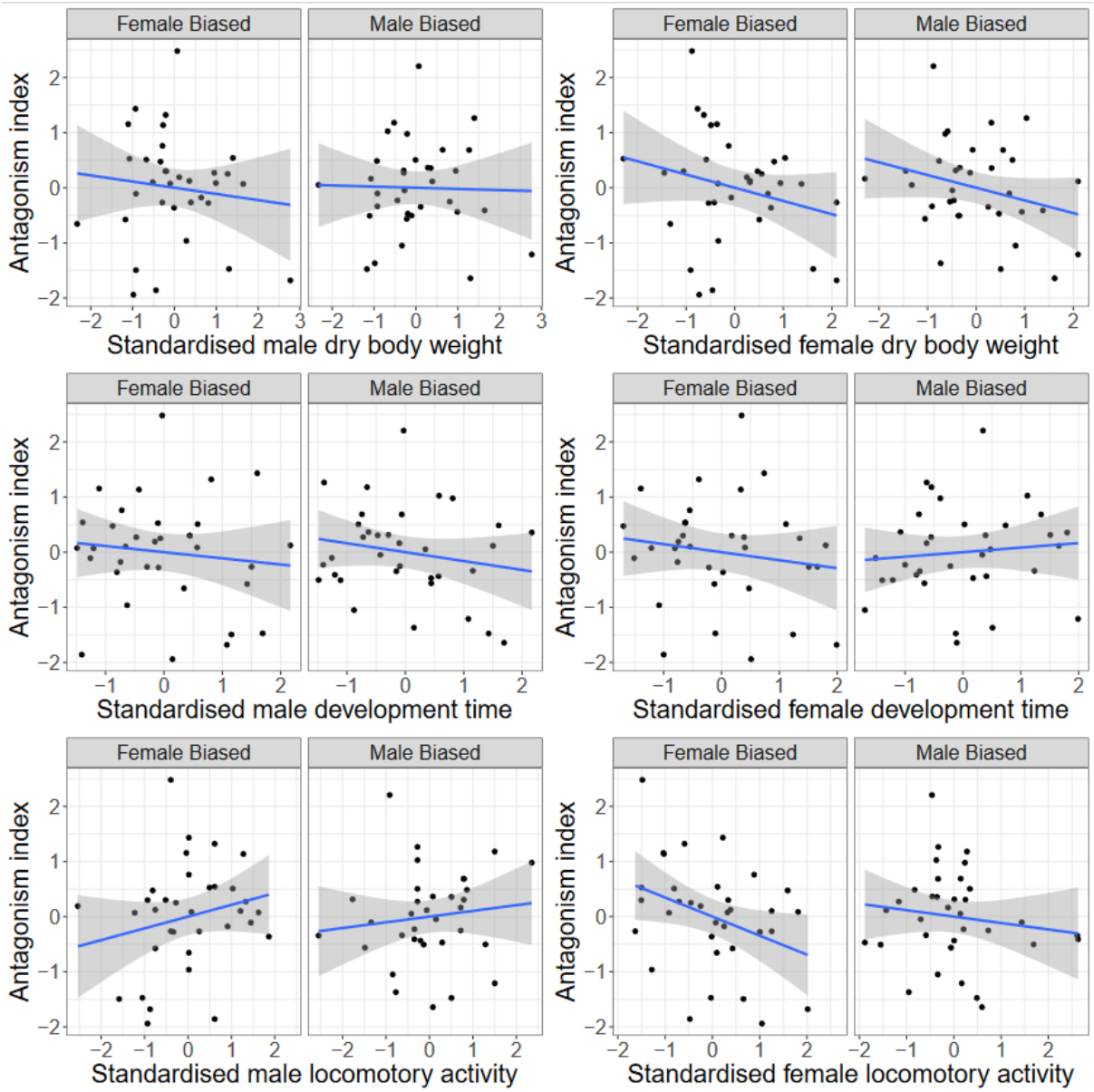

Figure 5a.4 Genetic correlations between antagonism index (AI) at male biased or female biased sex ratio and dry body weight, egg to adult development time and adult locomotory activity. The column on the left represents correlations for males, while the columns on the right represents correlations for females. First, second and third rows represent dry body weight, development time, and locomotory activity, respectively.

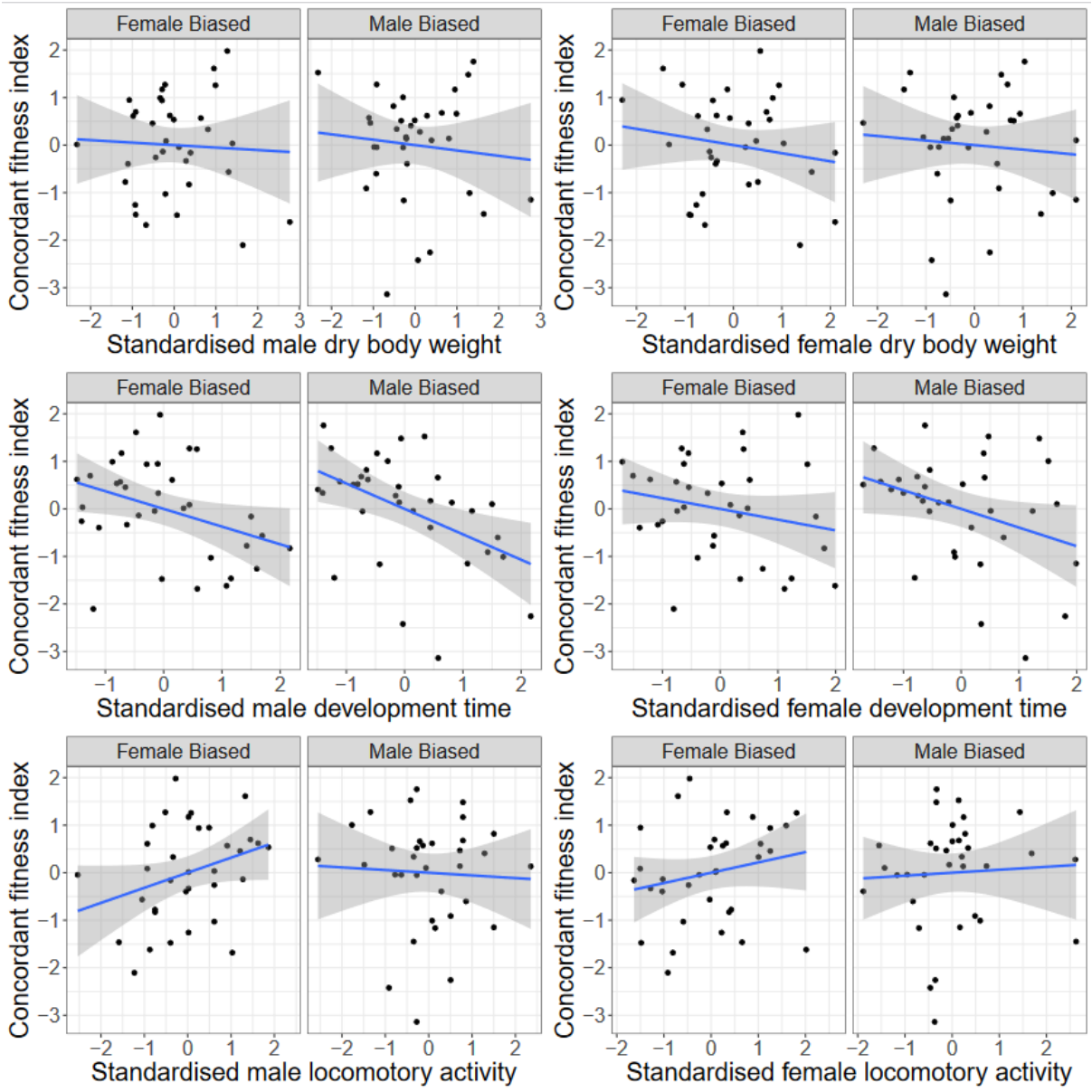

Figure 5a.5 Genetic correlations between sexually concordant fitness index at male biased or female biased sex ratio and dry body weight, egg to adult development time and adult locomotory activity. The column on the left represents correlations for males, while the columns on the right represents correlations for females. First, second and third rows represent dry body weight, development time, and locomotory activity, respectively.

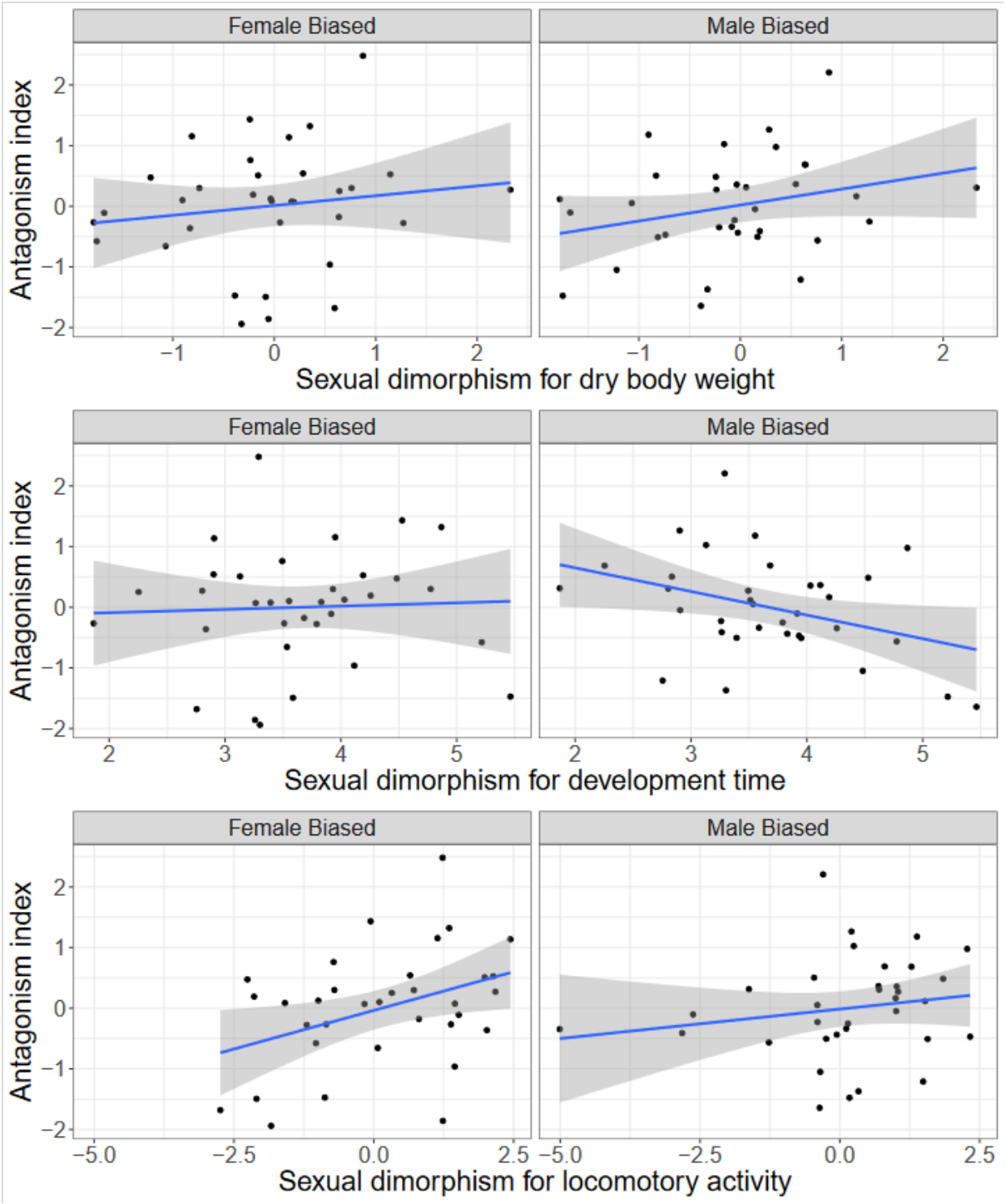

Figure 5a.6A Genetic correlations between antagonism index at male biased or female biased sex ratio and sexual dimorphism for dry body weight, egg to adult development time and adult locomotory activity. First, second and third rows represent dry body weight, development time, and locomotory activity, respectively.

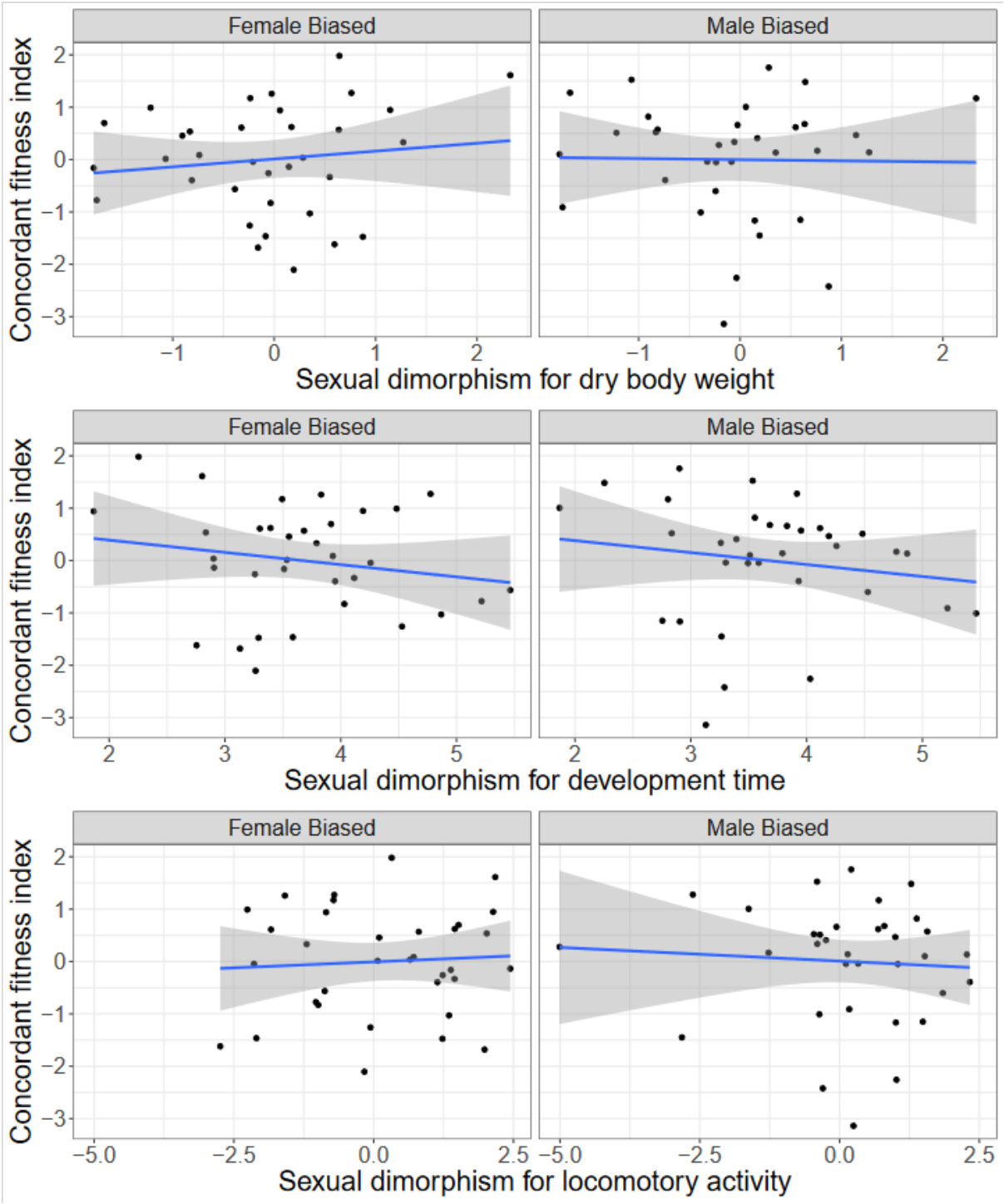

Figure 5a.6B Genetic correlations between sexually concordant fitness index at male biased or female biased sex ratio and sexual dimorphism for dry body weight, egg to adult development time and adult locomotory activity. First, second and third rows represent dry body weight, development time, and locomotory activity, respectively.

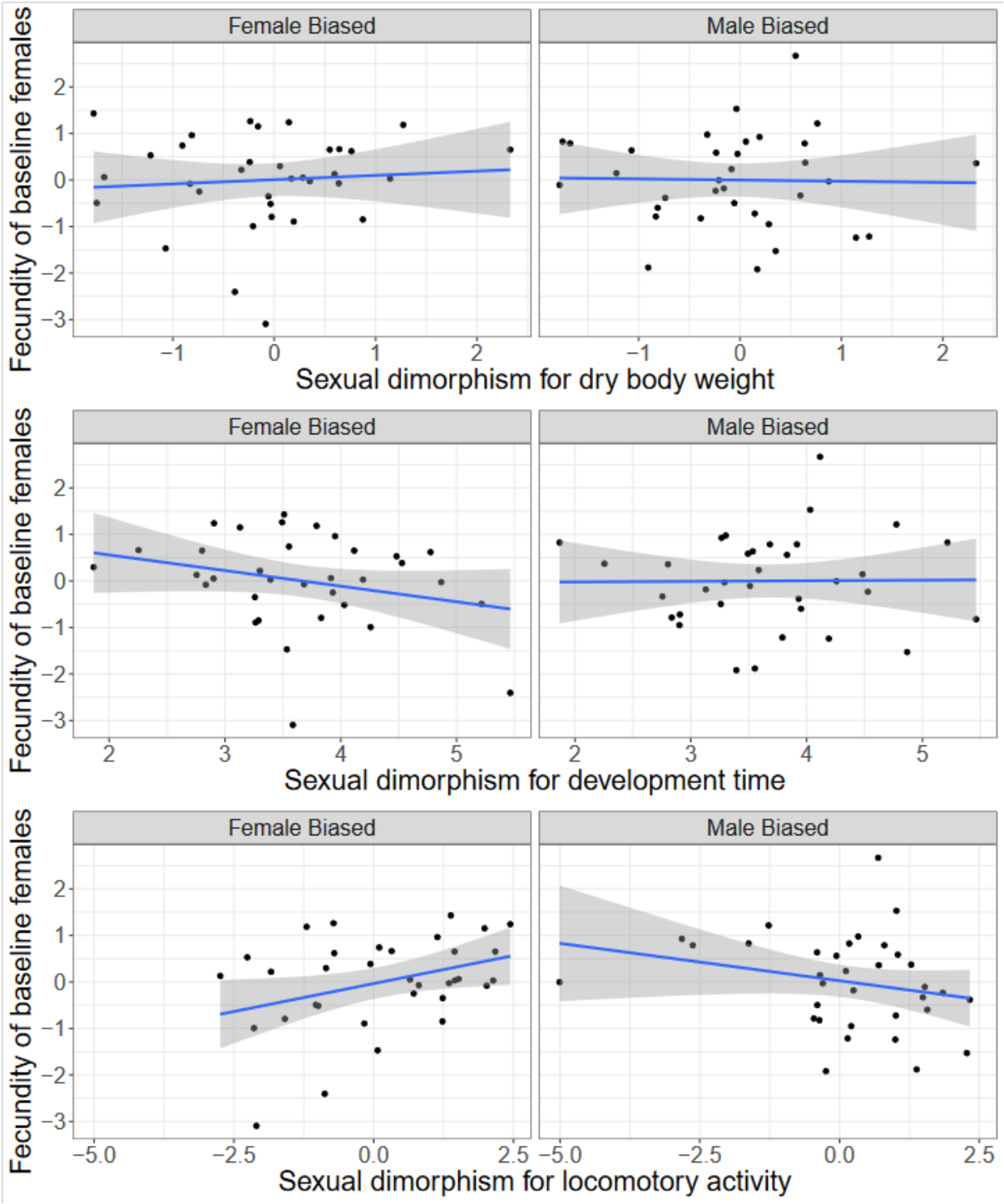

Figure 5a.6C Genetic correlations between fecundity of baseline females held with focal males at male biased or female biased sex ratio and sexual dimorphism for dry body weight, egg to adult development time and adult locomotory activity. First, second and third rows represent dry body weight, development time, and locomotory activity, respectively.

APPENDIX

Since locomotory activity lacked data from the equal sex ratio, all the analyses described above excluded fitness data from the equal sex ratio. In this appendix, I provide some figures representing sex-specific selection on dry body weight and development time, that includes equal sex ratio.

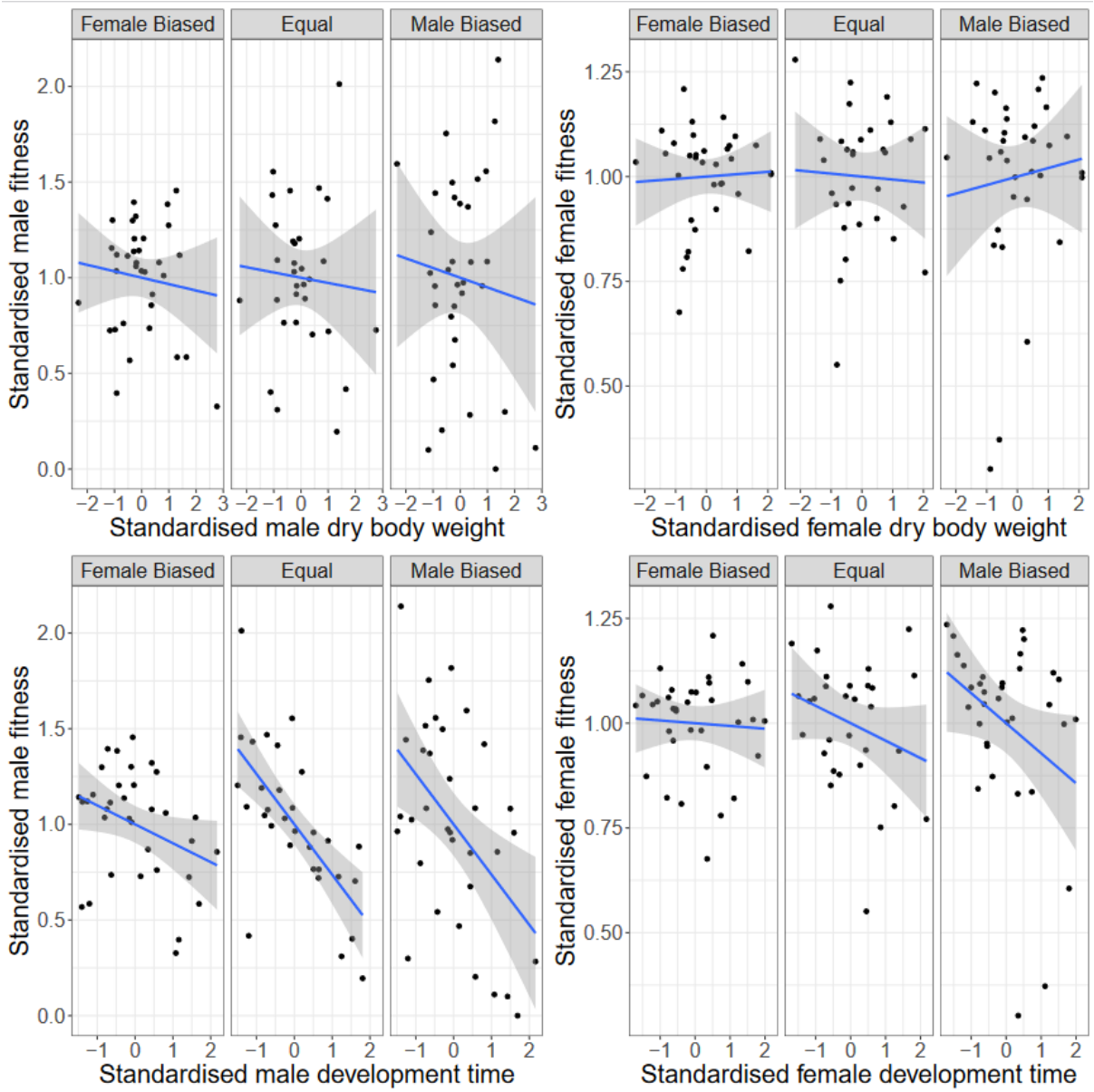

Figure 5a.7 Genetic correlations between relative male and female fitness at male biased, equal or female biased sex ratio and dry body weight and egg to adult development. The column on the left represents correlations for males, while the columns on the right represents correlations for females. First and second rows represent dry body weight and development time, respectively.

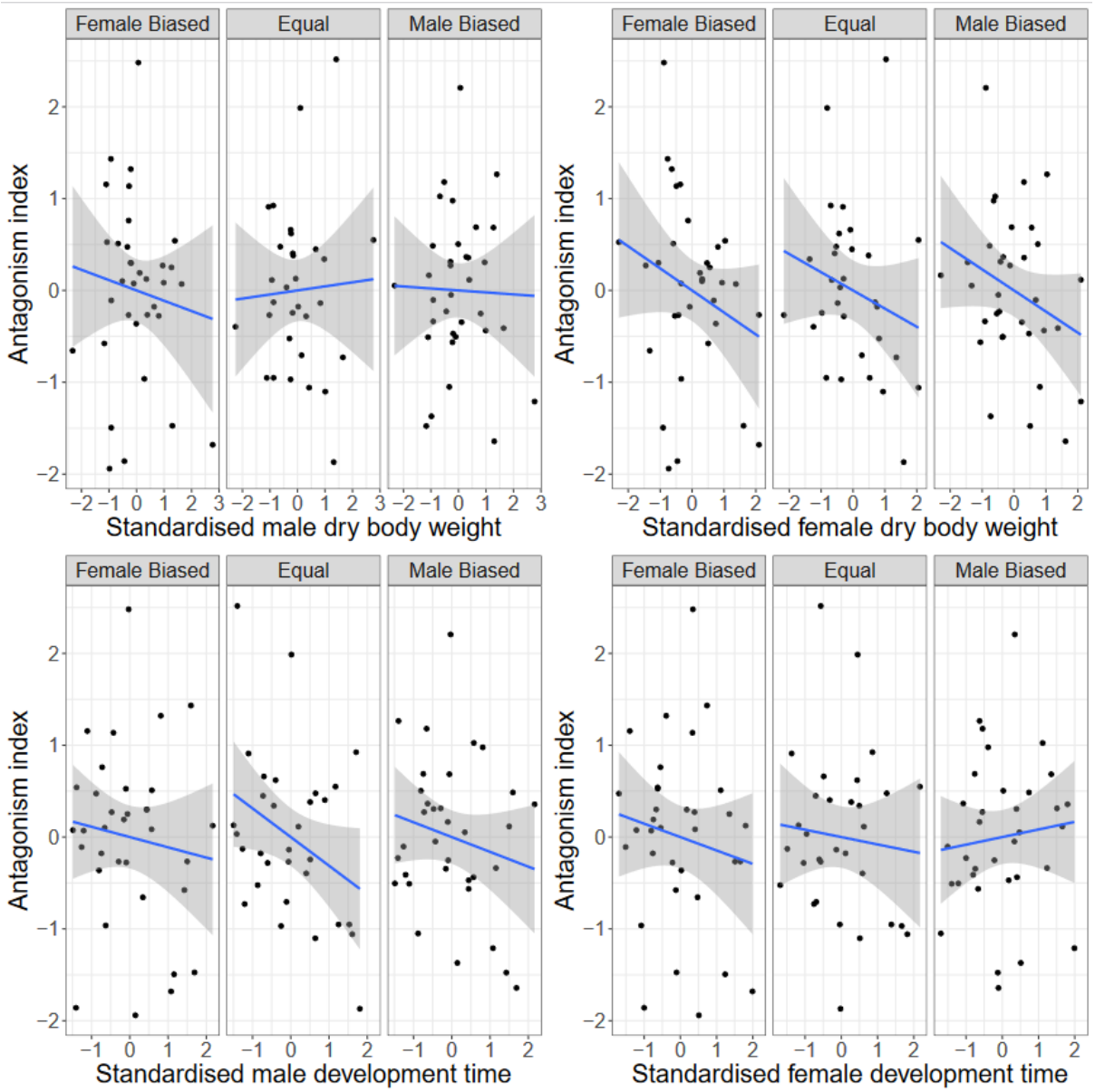

Figure 5a.8 Genetic correlations between antagonism index (AI) at male biased, equal or female biased sex ratio and dry body weight and egg to adult development. The column on the left represents correlations for males, while the columns on the right represents correlations for females. First and second rows represent dry body weight and development time, respectively.

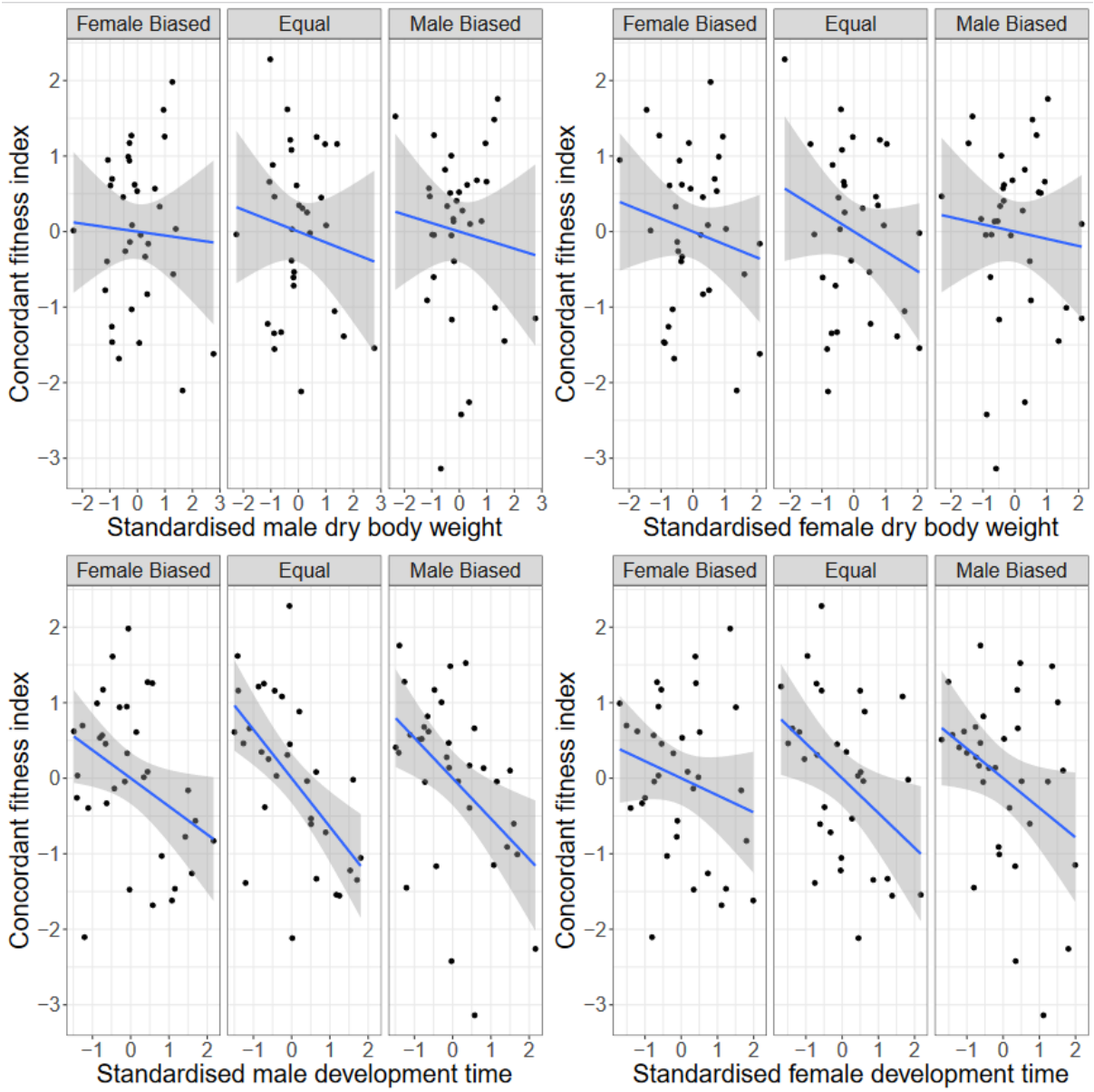

Figure 5a.9 Genetic correlations between sexually concordant fitness index at male biased, equal or female biased sex ratio and dry body weight and egg to adult development. The column on the left represents correlations for males, while the columns on the right represents correlations for females. First and second rows represent dry body weight and development time, respectively.

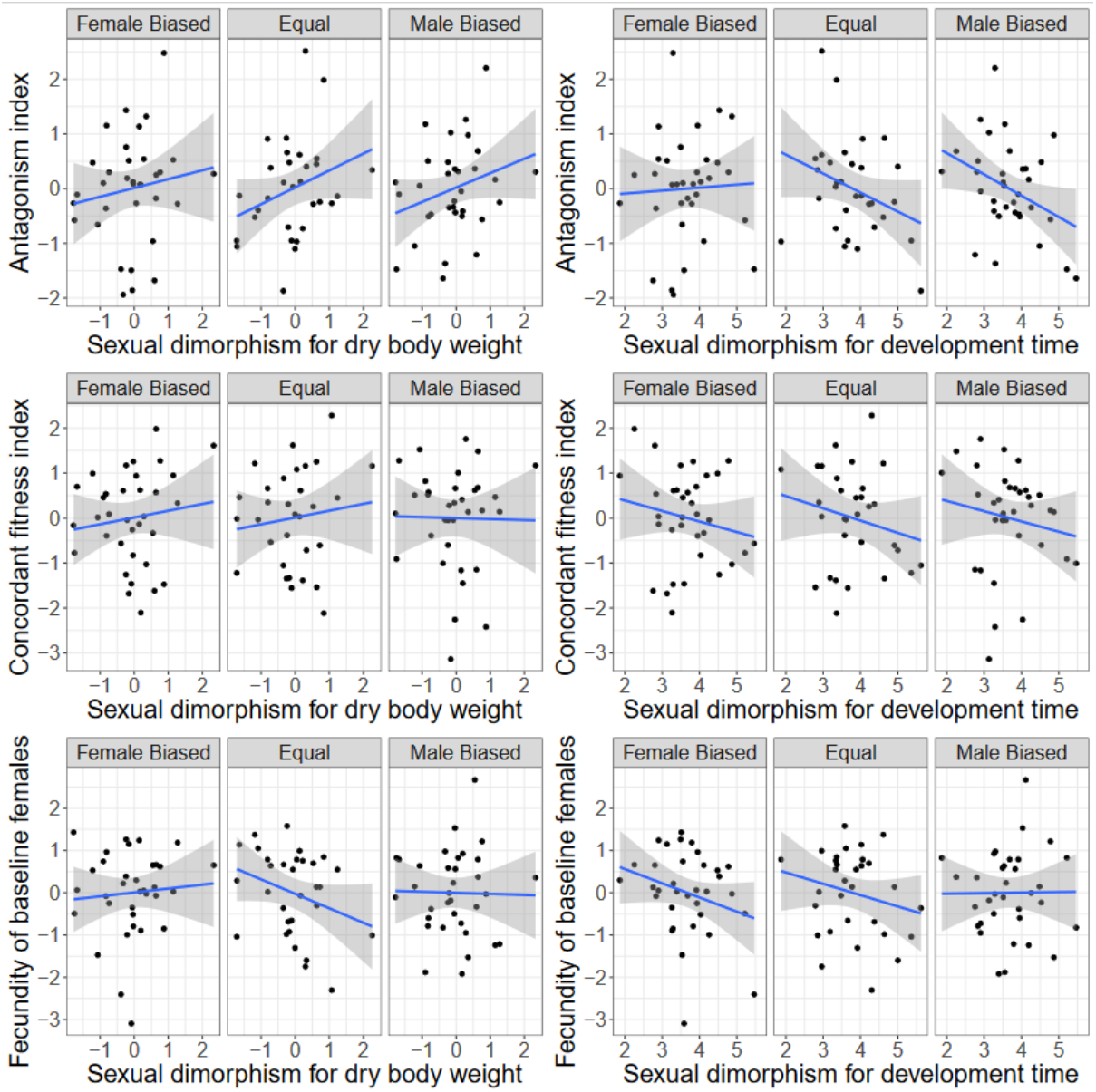

Figure 5a.10 Genetic correlations between antagonism index (first row), sexually concordant fitness index (second row) and fecundity of baseline females held with focal males (third row) at male biased, equal or female biased sex ratio and dry body weight (left column) and egg to adult development (right column).

Chapter 5b

Sexual dimorphism, sex-specific genetic architecture, and the nature of sex- and sex ratio-specific selection pertaining to wing shape

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INTRODUCTION

Selection often favours distinct sex-specific fitness optima for traits that can be defined for, and measured in, both males and females, leading to Intralocus Sexual Conflict (IaSC) (Bonduriansky and Chenoweth 2009). This creates conditions that are a necessary precondition to drive the evolution of sexual dimorphism that is so ubiquitous in the natural world. Both natural selection and sexual selection can drive the evolution of sexual dimorphism, provided additive genetic variation for sexual dimorphism is available. Nevertheless, independent evolution of males and females is severely constrained by the fact that genes that code for these traits in males either code for the traits in females as well, or are linked with genes that code for the traits in females (Lande 1980). This overlap in the underlying genetic machinery coding for shared traits in males and females is usually quantified by the parameter, intersexual additive genetic correlation, or r_{mf}. Across the natural world, with the exception of fitness related traits, r_{mf} is strongly positive for a variety of different kinds of traits, including behavioural, physiological, morphological as well as development related traits (Poissant et al. 2010).

In a seminal study, Lande (1980) developed a comprehensive quantitative genetic framework for how a bouquet of shared or sex-limited traits is expected to respond to sex-specific natural and sexual (frequency-dependent) selection. From an ecological perspective, Lande (1980) showed that even moderate sexual selection in one of the sexes has the potential to drive the other sex away from its sex-specific fitness optimum, reducing the average population fitness, and increasing the probability of extinction. Therefore, it becomes important to investigate the genetic architecture, as well as the patterns of sex specific selection on traits shared between males and females.

A large number of studies have investigated genetic architecture and sex-specific selection for individual traits. Patterns consistent with SA selection have been reported for multiple individual traits in many diverse species (Barson et al. 2015; Delph et al. 2011; Dutoit et al. 2018; Svensson et al. 2009;). However, predictions regarding how populations ought to respond to selection obtained from these studies employing univariate traits are not without their limitations. For example, correlations between the sexes and/or different traits may result in a situation where, a vast majority of the available additive genetic variation is distributed along one or few directions in the multivariate trait space, leaving little available additive genetic variation along some of the individual traits per se (Sztepanacz and Houle 2019). Second, a multivariate approach also likely results in a substantial reduction in measurement error, compared to a univariate approach, while using the same number of study specimens. A number of studies have attempted to investigate sex-specific genetic architectures of multivariate traits, including nine floral and leaf traits in *Silene latifolia* (Steven et al. 2007), cuticular hydrocarbons in *Drosophila serrata* (Gosden and Chenoweth 2014) and *Drosophila melanogaster* (Ingleby et al. 2014), and dewlap ornamentation in *Anolis sagrei* (Cox et al. 2017). Some studies have combined measurements of additive genetic (co)variances on multivariate traits with estimates of sex-specific selection acting on those traits. Working on isofemale lines of *Callosobrochus maculates,* Berger et al. (2016) investigated patterns of SA selection for multivariate morphological and life-history related traits. Holman and Jacomb (2017) measured the sex-specific genetic architecture and the degree of SA selection on three sexually dimorphic traits (development time, body mass, and elytra length) in *Tribolium castanaeum* across two distinct dietary environments.

Wing shape in *Drosophila* is an ideal system to experimentally investigate the framework established by Lande (1980) for a number of reasons. First, from a methodological point of view, with a large number of well-defined landmarks that are conserved across species (Houle et al. 2003; 2017), the *Drosophila* wing renders itself very convenient for comparative analysis using geometric morphometrics. Second, a number of studies have shown that wing shape in *Drosophila* has substantial additive genetic variation and that it can rapidly respond to selection (Pélabon et al. 2006; Menezes et al. 2013; Santos et al. 2004). Wing shape is also thought be a sexually selected trait with its association with mating success (Menezes et al. 2013; Trajković et al. 2021). There is also some evidence that wing shape could be under IaSC. For example, Sztepanacz and Houle (2019) investigated the sex-specific genetic architecture of wing shape in *D. melanogaster* and

detected strong cross-sex covariances for wing shape, which would impede independent evolution of the sexes. Consistent with this, Abbott et al. (2010) subjected replicate populations of *D. melanogaster* to male-limited evolution which relaxes sexually antagonistic (SA) selection, and found that wing shape evolved to become more masculinised in both sexes in the male-limited populations. Furthermore, this masculinisation of the wing shape was accompanied by an increase in male fitness, but a decrease in female fitness, a clear sign of SA selection. However, it is not clear if sexual selection in males contributes to this sexual antagonism over wing shape.

In this study, I use hemiclonal analysis (Abbott and Morrow 2011; Rice 1996) in a laboratory adapted population of *D. melanogaster* to investigate the genetic architecture and sex-specific selection on wing shape. I then ask whether the degree of sexual antagonism over wing shape is affected by the intensity of sexual selection and inter-locus sexual conflict (IeSC) in the population. I use adult sex ratio as the tool to modulate the intensity of sexual selection and IeSC in the population.

METHODS

Sampling and maintaining hemigenomes

Refer to Chapter 2 for the detailed protocol for sampling and maintaining hemigenomes from the LH population. Since five hemigenome lines were lost in an accident, this chapter only presents data from 34 hemigenome lines.

Generating experimental flies

I used flies generated for the development time and dry body weight experiment for wing morphometrics. Briefly, in order to generate experimental females, I crossed brown eyed males from each hemigenome line (heterozygous for the translocation and the target hemigenome) with virgin LH females. The red-eyed females emerging from these crosses were hemiclonal females that were used for wing morphometric analyses. In order to generate experimental males, I crossed brown eyed males from each hemigenome line with virgin DxLH females and collected the red eyed male progeny for wing morphometrics.

Wing morphometrics

For both males and females, I sampled five individuals from every rearing vial for each hemigenome line. For both males and females, I had two replicate assays, with each having

two replicate rearing vials for each hemigenome line. Thus, I sampled 20 (with some exceptions) individuals of each sex for each hemigenome line. From every individual I dissected out both left as well as the right wing, and mounted them on a glass slide with the help of a cover slip. I imaged each wing separately using Leica M205 C microscope. While imaging each wing, I also ensured that every image included a scale.

I used 11 wing landmarks (Figure 5b.1) used by Abbott et al. (2010) in their study. First, I constructed a "training set" by manually landmarking a subset of our sample images to train ML-morph to identify the landmark positions in the wing image. I used a training set containing 136 wing images that was landmarked manually at 11 homologous landmarks on the wing. These 136 images subset in the training set contained a random mixture of one left wing and one right wing from a male individual, as well as one left wing and one right wing from a female individual from each hemigenome line. This training set was considered ideal because it represents variation in wing shape with respect to sex, hemigenome line, and left or right wing assignment, that the main sources of variation in my sample set. I used the shape predictor function of the ML-Morph to train the algorithm to learn how I landmark the wing. Then, I tested the ability of ML-Morph to landmark wings accurately using the shape tester tool, where I compared automated landmarking by ML-morph to manual landmarks, which responded to 99.2% precision in the landmarking using automation in a test set build from the training set. Then, I applied shape predictor tool to landmark the whole image set. All landmarked images were later checked manually for any errors, and inconsistent landmarks across all images were corrected thereafter. It appeared that ML-morph was particularly error-prone in the case of images that were unclear, had smudges, or had debris interfering with the wing.

STATISTICAL ANALYSIS

All analyses were performed in R version 4.1.0

A. Geometric morphometric analyses

On the coordinate data for 11 landmarks obtained using ML-morph, I implemented the Procrustes algorithm using the "gpagen()" function in the R package "geomorph" (Adams and Otárola-Castillo 2013). This operation scales, rotates and translates every image to ensure that all the images overlap with respect to the 11 landmarks to the best possible degree. I then used this transformed landmark coordinate data to perform a principal component analysis (PCA) using the function "prcomp()". Using the function "lda()" in the

R package "MASS" (Ripley et al. 2022) I also performed a linear discriminant analysis (LDA) for sex, to identify the direction in which sexual dimorphism was most pronounced. I used the principal component and the linear discriminant scores for all wing images for subsequent analyses. Since the first three principal components explained more than 60% of the total variation (Figure 5b.2), I only included PC1, PC2 and PC3. To visualise, what variation along PC1, PC2, PC3, and LD1 means in terms of variation in wing shape, I plotted landmark profiles of wings with twice exaggerated lowest and highest scores along each of these axes.

B. Testing for the effect of sex and hemigenome line

To test for the effect of sex and hemigenome line, I fit the following linear mixed models using the R package "lmer4" and "lmerTest":

Y ~ Sex + (1|Replicate) + (1|Hemigenome line) + (1|Sex:Hemigenome line)

C. Sex-specific genetic architecture

In order to calculate sex-specific heritabilities as well as the intersexual genetic correlation for wing shape, I fit a Bayesian linear mixed model using Markov Chain Monte Carlo sampling methods implemented in the R package "MCMCglmm" separately for PC1, PC2, PC3 and LD1. I fit the following model for each of the four response variables:

$Y_{ijmno} \sim S_i + D + L_{ij} + V_m + F_n + \varepsilon_{ijmno}$, where Y_{ijmno} is the value (either PC1, PC2, PC3 or LD1) of the n^{th} individual fly of the m^{th} vial, for hemigenome line j, for sex i. S_i, models the fixed effect associated with sex, and D models the density of flies (estimated by counting the number of pupae) in the rearing vial. L_{ij} is a 2×2 matrix corresponding to the random effect of hemigenome line for each combination of sex and trait. L_{ij} is modelled to follow a multivariate normal distribution with mean 0, and the variance-covariance matrix given by sex-specific additive genetic variances and the additive genetic covariance between the sexes. F_n and V_n are matrices that model sex-specific variance associated with individual fly and vial, each modelled to follow a normal distribution with mean 0 and variance given by sex-specific individual fly related or rearing vial related variance, respectively. ε_{ijmn} represents the matrix of sex-specific residuals, with each term modelled to follow a normal distribution with mean 0 and variance given by sex- and trait-specific residual variance. For each model, I ran the simulation for 100000 iterations, out of which the first 25000 were discarded as "burn-in". In the next 75000 iterations every 50^{th} iteration

was sampled to create posterior distributions of our quantities of interest. I obtained posterior distributions of male and female heritabilities, as well as the intersexual additive genetic correlation (r_{mf}).

D. Linear and quadratic selection gradients on principal components and LD

Following Lande and Arnold (1983), I calculated sex- and sex ratio-specific linear and quadratic selection gradients by regressing relative fitness scores for hemigenome lines against the principal component scores, and separately for the LD1. In order to calculate the linear selection gradients, I fit the following linear models separately for each combination of sex and sex ratio:

Relative fitness ~ PC1 + PC2 + PC3

Relative fitness ~ LD1

In order to calculate quadratic and correlational selection gradients I fit the following models:

Relative fitness ~ PC1 + PC2 + PC3 + $(PC1)^2$ + $(PC2)^2$ + $(PC3)^2$ + (PC1 × PC2) + (PC1 × PC3) + (PC2 × PC3)

Relative fitness ~ LD1 + $(LD)^2$

I multiplied the coefficients corresponding to the quadratic terms by a factor of 2 to obtain quadratic selection gradients (Stinchcombe et al. 2008).

E. Angle between multivariate selection on males and females

I calculated the angle between the vectors representing the directions of multivariate selection acting on males and females in the space defined by PC1, PC2, and PC3. I used the following expression:

Angle = $cos^{-1}(\boldsymbol{\beta_m}.\boldsymbol{\beta_f}/(\sqrt{\boldsymbol{\beta_m^2}}\sqrt{\boldsymbol{\beta_f^2}})) \times 180/\pi$ where $\boldsymbol{\beta_m}$ and $\boldsymbol{\beta_f}$ are vectors corresponding to linear selection gradients in males and females.

I performed permutation tests to test if these angles were larger than expected if selection were *not* sex specific. I generated 1000 replicate data-sets by randomly permuting the sex of each data point. I calculated the angles between the selection gradients for males and females at each sex ratio for each of these 100 data-sets, to obtained the null-distribution

for the angles under the assumption that selection is not sex specific. Similarly, in order to obtain the null distribution for the angles if selection were not sex ratio-specific, I randomly permuted the sex ratios to generate 1000 replicate data-sets. The actual differences between the angles at different sex ratios were then tested against the null distribution defined by the 1000 replicate data-sets.

F. Genetic correlation between traits and SA or sexually concordant fitness variation

To investigate if wing shape was genetically correlated with SA fitness variation, following Berger et al. (2014) and Ruzicka et al. (2019), I calculated the "antagonism index" (AI) for each hemigenome line for each sex ratio, as described in Chapter 3. First, I calculated mean sex- and sex ratio-specific fitness for each hemigenome line as described above. I standardised these mean fitness scores for each combination of sex and sex ratio (mean = 0, variance = 1). Separately for each sex ratio, I rotated the coordinate system consisting of male fitness (y-axis) and female fitness (x-axis) 45^o in the anticlockwise direction using the following operation:

$\begin{pmatrix} \bar{W}_{C,i} \\ \bar{W}_{A,i} \end{pmatrix} = \begin{pmatrix} 1/\sqrt{2} & 1/\sqrt{2} \\ -1/\sqrt{2} & 1/\sqrt{2} \end{pmatrix} \begin{pmatrix} \bar{W}_{F,i} \\ \bar{W}_{M,i} \end{pmatrix}$, where $\bar{W}_{C,i}$ and $\bar{W}_{A,i}$ are the sexually concordant fitness component and AI respectively for the hemigenome line i for that sex ratio, and $\bar{W}_{F,i}$ and $\bar{W}_{M,i}$are the average female and male fitnesses respectively for the hemigenome line i for that sex ratio. I then regressed the sexually concordant fitness component and the AI for each sex ratio against standardised male and female PC1, PC2, PC3 and LD1.

G. Correlations between SD and SA variation and fecundity of mates

In order to calculate sexual dimorphism (SD) for wing shape, I first transformed sex-specific line means to have a variance of 1, while leaving the means unchanged. I calculated SD for each hemigenome line as the difference in the transformed LD1 values in males and the transformed LD1 value in females. I also calculated the line averages for the fecundity of females housed with males from various hemigenome lines in the locomotory activity experiment. I standardised these line averages for fecundity such that they had a mean 0 and variance 1. I then regressed, separately for each sex ratio, the AI, the sexually concordant fitness component and the standardised (mean = 0, variance = 1) fecundity scores for baseline females held with focal males against SD for wing shape.

RESULTS

A. Geometric morphometric analyses

The variance in wing shape data explained by various principal components in summarised in Figure 5b.2. The last four principal components did not explain any variance. This was a consequence of variables being lost during Procrustes fit. Twice exaggerated landmark profiles of wings with the lowest and the highest PC1 scores suggested that variation along PC1 was largely concentrated in the configurations of landmarks 4, 5, 6 and 7 (Figure 5b.3A). Similarly, wings with smaller values of PC2 appeared to be shorter and stubbier, while larger values of PC2 corresponded to elongated wings (Figure 5b.3B). Variation along PC3, to a large extent, translated to variation in the width of the distal end Figure 5b.3C). Twice exaggerated landmark profiles of wings with the smallest and the largest values of LD1 suggested that, among other aspects, female-like wings (small LD1) had narrower distal ends, while male-like wings (large LD1) had broader distal ends (Figure 5b.3D). Notice that variation in LD1 and PC3 are negatively correlated (Figure 5b.1, Figure 5b.2C-D). Visually, there appeared to be no separation between left and right wing for the first three principal components. However, the sexes separated out quite distinctly for PC3, and marginally so for PC1, but not for PC2 (Figure 5b.4).

B. Testing for the effect of sex and hemigenome line

The linear mixed models detected a statistically significant effect of sex for PC1, PC2, PC3, as well as for LD1. There was a statistically significant effect of wing for PC3, and the interaction between sex and wing was statistically significant for PC1, PC2 and LD1. There was a statistically significant effect of hemigenome line as well as its interaction with sex for PC1, PC2, PC3 and for LD1 (Table 5b.1).

C. Sex-specific genetic architecture

Table 5b.2 shows the outputs of the MCMCglmm models for separately for PC1, PC2, PC3 and LD1. Male heritability estimates were higher than females for PC1 (male: 1.2715, 95% credible intervals (CI) = [1.072029, 1.501815], female = 0.9420461, 95% CI = [0.6995802, 1.197113]), PC2 (male: 1.034835, 95% CI = [0.7917119, 1.296076], female: 0.8928031, 95% CI = [0.6389776, 1.146203]) and PC3 (male: 0.9195002, 95% CI = [0.6616094, 1.179975], female: 0.724614, 95% CI = [0.4794641, 1.006331]), but not for LD1 (male: 0.4711856, 95% CI = [0.2718781, 0.6841523], female: 0.4951413, 95% CI = [0.2789619,

0.7124838]). I also detected significantly positive intersexual genetic correlations for PC1 (0.8923091, 95% CI = [0.8013813, 0.9630189]), PC2 (0.7040355, 95% CI = [0.5135586, 0.887593]), PC3 (0.7347744, 95% CI = [0.5294574, 0.9091386]), and LD1 (0.5091726, 95% CI = [0.1847809, 0.8184318]) (Figure 5b.5). Note that rmf for the axis along which the sexes were best separated was the lowest.

D. Linear and quadratic selection gradients on principal components and LD

The linear, quadratic and correlational selection gradients are summarised in Table 5b.3. None of the linear selection gradients were statistically significant. However, a few interesting patterns stood out. In males at the male biased and equal sex ratios there appeared to be directional selection for smaller LD1 scores, i.e., for more "female-like" wings. There appeared to be some evidence for SA selection on PC2 at the male biased sex ratio. At the male biased sex ratio, the selection gradient on PC2 was positive for females, but negative for males. This trend was not apparent in the female biased or equal sex ratios.

None of the quadratic selection gradients were statistically significant, except for PC3 appeared to be under stabilising selection in males at the male biased sex ratio. None of the correlational selection gradients were statistically significant either.

E. Angle between multivariate selection on males and females

The angles between the directions of selection in males and females at male biased, equal and females biased sex ratio were 134.3151, 138.6842 and 66.59779 degrees respectively. respectively. None of these angles were larger than expected under the null hypothesis that selection was sex-specific (male biased: p =0.274; equal: p = 0.349; female biased = 0.983) based on our permutation tests. The difference in these angles at equal and female biased sex ratios was also not statistically significant, based on our permutation test by sex (p = 0.164).

F. Genetic correlation between traits and SA or sexually concordant fitness variation fitness variation

The genetic correlations between AI and PC1, PC3 and LD1 were not statistically significant for either sex, at any of the three sex ratios (Table 5b.4). However, at the male biased sex ratio, PC2 was negatively genetically correlated with AI in both males and females, consistent with our finding that selection gradients on PC2 had opposite signs in the two sexes at the male biased sex ratio.

Sexually concordant fitness index was not genetically correlated with PC1, PC2 or PC3 in both sexes and at each of the three sex ratios (Table 5b.5). However, I found a significantly negative genetic correlation between male LD1 and sexually concordant fitness index at the male biased sex ratio.

G. Correlations between SD and SA variation and fecundity of mates

The outputs of the linear models for antagonism index, sexually concordant fitness index, the fecundity of baseline female held with focal males, with sexual dimorphism as the explanatory variable, are summarised in Table 5b.6. None of these correlations were statistically significant, except for the model for sexually concordant fitness index at equal sex ratio, where more dimorphic lines had lower sexually concordant fitness indices (Figure 5b.9).

DISCUSSION

In this chapter I investigated the sex-specific genetic architecture for wing shape and the nature of sex-specific selection acting on wing shape at male biased, equal and female biased sex ratios. My main findings were as follows:

(1) I found appreciable additive genetic variation for wing shape along multiple directions (PC1, PC2, PC3, LD1). Furthermore, along each of these directions, there was a strongly positive intersexual genetic correlation, suggesting that the sexes would not be free to evolve independently in response to SA selection.

(2) There was statistically significant effect of sex along all the axes I investigated. However, sexes were well-separated along PC3, and of course, LD1. The effect of wing (left vs right) was also statistically significant. However, the magnitude of this difference appeared to be small.

(3) There was compelling evidence of SA selection operating on PC2 (which explained about 16% of the total variation in wing shape), but only at the male biased sex ratio.

(4) There was no evidence to suggest that sexual dimorphism in wing shape was associated with more male mate harm caused by males.

Consistent with previous studies investigating the sex-specific genetic architecture of *D. melanogaster* wing shape (Sztepanacz and Houle 2019), I found substantial additive

genetic variation in both sexes. However, my heritability estimates were fairly high. For PC1, the estimate of male heritability was well above 1. Since all hemiclones belonging to the same hemigenome line share half of their entire genome, while calculating heritabilities I had multiplied the variance associated with hemigenome line by 2. The fact that these heritability estimates are large is likely a result of variance due to other factors that were not controlled for by my experimental design, being misattributed to variance associated with hemigenome line. This could have happened in several ways. First, it is possible that my estimates of heritability were inflated by variance due to maternal effects, or variance between hemiclonal families as a result of sampling error in selecting mothers of experimental flies from the LH (or DxLH) population. Additionally, some environmental effects associated with rearing hemiclonal flies together could also get attributed to variance associated with hemigenome line. For the X chromosome, the relatedness among hemiclone males of the same hemigenome line was 1. My experimental design did not allow me to partition X-linked variation from autosomal variation. Therefore, multiplying *entire* variance associated with hemigenome line by 2, would inflate estimates of heritability, if there was appreciable X-linked additive genetic variation for wing shape. As a result, these heritability estimates represent upper bounds of heritabilities.

I also detected a clear signature of IaSC over certain aspects of wing shape. Firstly, there was a strong intersexual genetic correlation for PC1, PC2, PC3 and LD1. Secondly, PC2 showed a clear sign of being under SA selection, with selection gradients having opposite signs, but only at the male biased sex ratio. This was confirmed by the statistically significant genetic correlation between PC2 scores in both sexes and the antagonism index at the male biased sex ratio. This suggests that, while IaSC and IeSC may be mutually exclusive in their preliminary mathematical formalisms (Gavrilets et al. 2001; Kidwell et al. 1977), they may actually interact, and in some cases may reinforce each other. From an empirical point of view, though, this is not such a surprising result. A vast majority of studies investigating IaSC, have detected signatures of SA selection in reproduction related traits (reviewed in Cox and Calsbeek (2009)). Strengthening sexual selection, therefore, should lead to a greater separation of sex-specific fitness optima, resulting in stronger IaSC over that trait. Stronger sexual antagonism for wing shape at the male biased sex ratio, however, is not consistent with my findings in Chapter 3 and Chapter 5a. I had shown that the signals of IaSC (intersexual genetic correlation for fitness and the proportion of SA fitness variation) were *weaker* at the male biased sex ratio. I had also reported strong

sexually concordant selection on development time at the male biased sex ratio (Chapter 5a). Taken together, these two results seem to suggest that the interaction between IaSC and IeSC may unfold in complicated ways, with the two reinforcing each other in some cases (e.g., wing shape), while strengthening may cause the amelioration of the other in some other cases (e.g., development time). Some studies have suggested that IaSC and IeSC may interact if sexual dimorphism leads to trait exaggeration in males such that these males now cause greater mate harm to females (Berger et al. 2016; Pennell and Morrow 2013). I found no evidence for this hypothesis, as baseline females held with males from the most dimorphic lines showed no signs of any reduction in their fecundity.

Exaggerated landmark profiles of wings with the smallest and the highest PC2 scores suggested that, at the male biased sex ratio, females were under selection to evolve elongated wings, while males were under selection to evolve shorter, stubbier wings. This is in contrast to the results of Menezes et al. (2013), who selected flies for elongated wings and showed at male mating success improved. One of the reasons for this inconsistency could be that the selection protocol employed by Menezes et al. (2013) failed to account for body size variation. Every generation they would select 50 females and select 10 females with the lowest width:length ratio for the wings in the selection regime for longer wings (L), and 10 females with the highest width:length ratio for the wings in the selection regime for rounded wings (R). If, for some reason, variance in wing length is larger than variance in wing width, their protocol for selection elongated wings, could be selecting for *larger* wings. In fact, they found that L males were indeed larger than R males. While the relationship between mating success and body size is complex, some studies have reported that larger males enjoy greater mating success in *Drosophila* (Bangham et al. 2002; Partridge and Farquhar 1983; Partridge et al. 1987). By contrast, before analysing wing shape using 11 distinct landmarks, the Procrustes algorithm scaled every wing to a centroid size of 1. Additionally, I used competitive fertilization success as a measure of male fitness, which is a composite measure of mating and fertilization success. Therefore, especially given that the relationship between mating and fertilization success is somewhat complex (De Nardo et al. 2021), it is not entirely surprising that my results are not consistent with those of Menezes et al. (2013).

My results are also partially consistent with the findings of Abbott et al. (2010). Similar to my observations (Figure 5b.3D), they too detected that sexual dimorphism in wing shape

was largely associated with male – female differences in the relative width of the proximal vs the distal part of the wing, with males having broader distal ends. Additionally, they reported that male-limited evolution resulted in the masculinization (i.e., broader distal parts, and narrower proximal parts) of both male and female wings, albeit via a slightly different transformation in the arrangement of wing landmarks compared to the one required for sexual dimorphism (see Figure 1 in Abbott et al. (2010)). This implies that in the ancestral population of their experimental evolution lines there was SA genetic variation for wing shape roughly along the axis corresponding to sexual dimorphism (i.e., relative width of proximal vs distal parts of the wing). While I, too, detected evidence of IaSC over wing shape (although only at male biased sex ratio), it is important to note that the axis of wing shape variation along which this sexual antagonism was distributed was entirely different from the one for which Abbott et al. (2010) found evidence of IaSC. I found no evidence of SA selection operating on directions in wing shape variation that corresponded to sexual dimorphism (i.e., LD1 and PC3). However, I found that the component of wing shape that was the least sexually dimorphism, i.e., PC2, was under IaSC. Physically, this translated to an entirely different axis of wing shape variation, one that is dominated by variation in the degree of roundedness vs elongation (Figure 5b.2B). Interestingly, the LH population used in this thesis is the direct descendant of the LH_M population from which Abbott et al. (2010) derived their male-limited lines. In fact, Chippindale et al. (2001) had previously detected a strongly *negative* intersexual genetic correlation for adult reproductive fitness in the LH_M population. In contrast, I reported in Chapter 3 that the intersexual genetic correlation for adult reproductive fitness in the LH population in our laboratory was significantly greater than 0 at each of the three sex ratios. This result coupled with the finding that the target of SA selection on wing shape in the LH population seems to have changed points towards a scenario where the genetic architecture of the LH population has evolved extensively in the last two decades (> 500 generations), perhaps, in response adaptation to the laboratory environment.

Intersexual genetic correlations (r_{mf}) can severely constrain the evolution of sex-specific adaptation and sexual dimorphism, despite sex-specific/SA selection (Lande and Arnold 1983). Sex-specific genetic architecture can help the sexes reach their respective sex-specific fitness optima, leading to sexual dimorphism, resolving IaSC in the process (Bonduriansky and Chenoweth 2009). Consistent with these ideas, I first found that the component of wing shape where the sexes were best separated, i.e., the linear discriminant

axis for sex, had the weakest r_{mf}. This is in agreement with Poissant et al. (2010) who conducted a meta-analysis of r_{mf} and sexual dimorphism and found a negative correlation between the two. Next, I found evidence of IaSC over the component of the wing shape, i.e., PC2, along which there was little or no sexual dimorphism, suggesting that SA selection, coupled with a strong r_{mf}, might have constrained the evolution of sexual dimorphism along this axis.

Lastly, my estimates of male and female heritabilities allow me to speculate about the potential role of X-linked genetic variation in wing shape. Some studies have shown that the heterogametic sex should exhibit greater genetic variation for traits, primarily because the hemizygosity of the X or the Z chromosome in the heterogametic sex allows for the expression of recessive alleles (Connallon 2010; Reinhold and Engqvist 2013). In my system, if there was substantial X-linked variation for wing shape, it should be reflected in greater heritability for wing shape in males than in females. Furthermore, as I have described above, the protocol used for calculating heritabilities from MCMCglmm models, also tended to inflate X-linked genetic variation. Therefore, greater heritabilities in males than in females would be consistent with, though certainly not direct evidence of, substantial X-linked variation for that component. In this study, I found that male heritability for PC1 for wing shape was significantly greater than the corresponding female heritability. Male heritabilities for PC2 and PC3 were also larger than the corresponding female heritabilities, but not significantly so. On the other hand, for LD1, the direction of strongest sexual dimorphism, male heritability was marginally lower than female heritability. Although this cannot count as direct evidence, these findings seem to suggest that the most sexually dimorphic component of wing shape has depleted most of its X-linked genetic variation. This is consistent with the theoretical expectation that X chromosomes are more conducive for the resolution of IaSC (leading to sexual dimorphism), via the fixation of one of the alleles at SA loci in linkage with sex-specific modifiers of gene expression (Connallon and Clark 2010). A straightforward way to experimentally test for the depletion of X-linked variation related to wing shape would be to isolate a panel of X chromosomes and a panel of autosomes form the LH population using methods described by Griffin et al. (2016). Investigating the sex-specific genetic architecture of wing shape in these lines would provide useful insights in the relative contribution of X chromosomes and autosomes at maintaining genetic variation for various components of wing shape.

CONCLUSION

In this chapter, I investigated the sexual dimorphism, sex-specific genetic architecture and sexual antagonism for wing shape. I found strong sexual dimorphism for wing shape. Male wings had much broader distal ends than female wings. I also found substantial additive genetic variation in both sexes for all components of wing shape I investigated. There were statistically significant intersexual genetic correlations for all components of wing shape under investigation. Interestingly, the intersexual genetic correlation was the weakest along the direction where males and females were the best separated. I found compelling evidence for sexual antagonism for PC2, but only at male biased sex ratio, where males with shorter and stubbier wings, but females with elongated wings enjoyed fitness benefits. This suggests that strengthening sexual selection and IeSC led to aa stronger signal of IaSC over wing shape. Lastly, my estimates of heritabilities were typically higher in males than in females, except for LD1, the axis of strongest sexual dimorphism. It is likely that this indicates a role of the X chromosome in the evolution of sexual dimorphism, although more experimental validation is required.

Outputs of mixed model ANOVAs with Satterthwaite's method						
(A) Fixed effects						
PC1	**Sum Sq**	**Mean Sq**	**NumDF**	**DenDF**	**F value**	**Pr(>F)**
Sex	0.0063	0.0063	1	30.3600	97.6462	**<0.0001**
Wing	0.0000	0.0000	1	2456.6500	0.1551	0.6937
Sex:Wing	0.0000	0.0000	1	2456.6400	0.2702	**0.6032**
PC2	**Sum Sq**	**Mean Sq**	**NumDF**	**DenDF**	**F value**	**Pr(>F)**
Sex	0.0004	0.0004	1	25.2000	8.8674	**0.0063**
Wing	0.0004	0.0004	1	2456.0000	8.8336	0.0030
Sex:Wing	0.0003	0.0003	1	2456.0000	7.0529	**0.0080**
PC3	**Sum Sq**	**Mean Sq**	**NumDF**	**DenDF**	**F value**	**Pr(>F)**
Sex	0.0067	0.0067	1	26.9300	241.8842	**<0.0001**
Wing	0.0002	0.0002	1	2456.5000	5.9295	**0.0150**
Sex:Wing	0.0001	0.0001	1	2456.5000	3.2346	0.0722
LD1	**Sum Sq**	**Mean Sq**	**NumDF**	**DenDF**	**F value**	**Pr(>F)**
Sex	829.6500	829.6500	1	30.3100	1097.2835	**<0.0001**
Wing	0.0300	0.0300	1	2456.7100	0.0427	0.8364
Sex:Wing	2.4900	2.4900	1	2456.7100	3.2934	**0.0697**
(B) Random effects						
PC1	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	8	8492.9	-16970			
(1|Replicate)	7	8492.7	-16971	4.47E-01	1	0.5038
(1|Hemigenome line)	7	8470.6	-16927	4.47E+01	1	**<0.0001**
(1|Sex:Hemigenome line)	7	8428.1	-16842	1.30E+02	1	**<0.0001**
PC2	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	8	8986.2	-17956			
(1|Replicate)	7	8974.6	-17935	2.31E+01	1	**<0.0001**
(1|Hemigenome line)	7	8975.4	-17937	2.16E+01	1	**<0.0001**
(1|Sex:Hemigenome line)	7	8867	-17720	2.38E+02	1	**<0.0001**
PC3	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	8	9554.8	-19094			
(1|Replicate)	7	9550.2	-19087	9.228	1	**0.0024**
(1|Hemigenome line)	7	9542.7	-19071	24.278	1	**<0.0001**
(1|Sex:Hemigenome line)	7	9476.9	-18940	1.56E+02	1	**<0.0001**
LD1	**npar**	**logLik**	**AIC**	**LRT**	**Df**	**Pr(>Chisq)**
<none>	8	-3328.5	6673.1			
(1|Replicate)	7	-3328.9	6671.8	0.768	1	0.3807
(1|Hemigenome line)	7	-3333.1	6680.2	9.085	1	**0.0026**
(1|Sex:Hemigenome line)	7	-3392.3	6798.6	127.516	1	**<0.0001**

Table 5b.1 The output of linear mixed effects models for PC1, PC2, PC3 and LD1. P values less than 0.05 are shown in bold.

Outputs of MCMCglmm models				
		Estimate	**Lower CL**	**Upper CL**
PC1	**Female heritability**	0.9420	0.6996	1.1971
	Male heritability	1.2715	1.0720	1.5018
	Intersexual genetic correlation	0.8923	0.8014	0.9630
	Fixed effect of density	-0.0002383	-0.0009541	0.0004902
PC2	**Female heritability**	0.8928	0.6390	1.1462
	Male heritability	1.0348	0.7917	1.2961
	Intersexual genetic correlation	0.7040	0.5136	0.8876
	Fixed effect of density	-0.0000815	-0.0009606	0.0008587
PC3	**Female heritability**	0.7246	0.4795	1.0063
	Male heritability	0.9195	0.6616	1.1800
	Intersexual genetic correlation	0.7348	0.5295	0.9091
	Fixed effect of density	0.0003532	-0.0005847	0.0014816
LD1	**Female heritability**	0.4951	0.2790	0.7125
	Male heritability	0.4712	0.2719	0.6842
	Intersexual genetic correlation	0.5092	0.1848	0.8184
	Fixed effect of density	-0.0012192	-0.0022213	-0.0000389

Table 5b.2 Summary of sex-specific heritabilities, the intersexual genetic correlation and the fixed effect of rearing density on PC1, PC2, PC3, and LD1 obtained using Bayesian mixed effects models implemented in MCMCglmm.

(A) Linear selection gradients					
		Females		Males	
		Estimate	Standard error	Estimate	Standard error
Female biased sex ratio	PC1	-0.0070	0.0256	-0.0104	0.0570
	PC2	0.0069	0.0213	-0.0330	0.0522
	PC3	-0.0079	0.0259	0.0298	0.0569
	LD1	0.0106	0.0204	-0.0335	0.0506
Equal sex ratio	PC1	0.0024	0.0341	0.0065	0.0756
	PC2	-0.0002	0.0284	0.0174	0.0692
	PC3	0.0328	0.0344	0.0405	0.0755
	LD1	-0.0059	0.0277	-0.1108	0.0641
Male biased sex ratio	PC1	0.0413	0.0451	0.0271	0.1041
	PC2	0.0569	0.0376	-0.1169	0.0954
	PC3	0.0461	0.0456	0.0186	0.1040
	LD1	-0.0246	0.0374	-0.1362	0.0912
(B) Quadratic selection gradients					
		Females		Males	
		Estimate	Standard error	Estimate	Standard error
Female biased sex ratio	PC1	-0.0671	0.0465	0.0782	0.0792
	PC2	-0.0836	0.0335	-0.0914	0.0761
	PC3	0.0447	0.0656	-0.1115	0.1225
	LD1	-0.0208	0.0437	0.0571	0.1147
Equal sex ratio	PC1	-0.0029	0.0683	0.0475	0.1125
	PC2	-0.0518	0.0491	-0.0590	0.1083
	PC3	0.0715	0.0962	-0.1077	0.1742
	LD1	-0.0389	0.0593	-0.0415	0.1456
Male biased sex ratio	PC1	-0.0212	0.0897	-0.0259	0.1382
	PC2	-0.0818	0.0645	-0.1277	0.1330
	PC3	0.1415	0.1263	**-0.5143**	**0.2140**
	LD1	-0.0389	0.0804	0.0212	0.2073
(C) Correlational selection gradients					
		Females		Males	
		Estimate	Standard error	Estimate	Standard error
Female biased sex ratio	PC1-PC2	-0.0078	0.0495	-0.0874	0.0881
	PC1-PC3	0.0061	0.0506	0.0813	0.0986
	PC2-PC3	0.0560	0.0340	0.0745	0.0929
Female biased sex ratio	PC1-PC2	-0.0161	0.0726	-0.0788	0.1253
	PC1-PC3	0.0358	0.0743	0.0944	0.1402
	PC2-PC3	0.0804	0.0498	0.0070	0.1321
Male biased sex ratio	PC1-PC2	-0.0284	0.0954	0.0584	0.1539
	PC1-PC3	0.0896	0.0975	-0.0919	0.1722
	PC2-PC3	0.1119	0.0654	0.1177	0.1622

Table 5b.3 Summary of (A) linear and (B) quadratic selection gradients on PC1, PC2, PC3 and LD1, and (C) correlational selection gradients between PC1, PC2 and PC3. Values indicated in bold correspond to selection gradients significantly different from 0.

Linear models for SA fitness variation ~ trait					
(A) Female biased sex ratio					
	Trait	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Females	PC1	0.0476	0.1715	0.2780	0.7830
	PC2	-0.250	0.1659	-1.5040	0.1420
	PC3	0.1281	0.1702	0.7530	0.4570
	LD1	-0.1837	0.1686	-1.0890	0.2840
Males	PC1	-0.0677	0.1713	-0.3950	0.6950
	PC2	-0.2245	0.1671	-1.3440	0.1880
	PC3	0.0024	0.1717	0.0140	0.9890
	LD1	0.0693	0.1713	0.4050	0.6890
(B) Equal sex ratio					
	Trait	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Females	PC1	0.1005	0.1550	0.6480	0.5210
	PC2	-0.1283	0.1543	-0.8310	0.4120
	PC3	-0.0678	0.1555	-0.4360	0.6660
	LD1	0.0125	0.1560	0.0800	0.9370
Males	PC1	0.0345	0.1559	0.2210	0.8260
	PC2	-0.0902	0.1552	-0.5810	0.5650
	PC3	-0.1878	0.1524	-1.2320	0.2270
	LD1	4.16E-02	0.1558	0.2670	0.7910
(C) Male biased sex ratio					
	Trait	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Females	PC1	0.0908	0.1466	0.6190	0.5400
	PC2	-0.3873	0.1306	-2.9660	**0.0057**
	PC3	0.0275	0.1474	0.1870	0.8530
	LD1	-0.0883	0.1466	-0.6020	0.5510
Males	PC1	-0.0008	0.1475	-0.0060	0.9950
	PC2	-0.3268	0.1357	-2.4090	**0.0219**
	PC3	-0.1939	0.1434	-1.3520	0.1860
	LD1	0.0436	0.1473	0.2960	0.7690

Table 5b.4 Summary of linear models for the antagonism index at the three sex ratios with male and female PC1, PC2, PC3 and LD1. P values less than 0.05 are shown in bold.

Linear models for sexually concordant fitness variation ~ trait					
(A) Female biased sex ratio					
	Trait	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Females	PC1	0.0176	0.182	0.097	0.924
	PC2	-0.1532	0.1797	-0.8520	0.4000
	PC3	0.0682	0.1813	0.3760	0.7090
	LD1	-0.0541	0.1815	-0.2980	0.7670
Males	PC1	-0.0303	0.1816	-0.1670	0.8690
	PC2	0.0597	0.1814	0.3290	0.7440
	PC3	0.1718	0.1791	0.9590	0.3450
	LD1	-0.2335	0.1770	-1.3190	0.1960
(B) Equal sex ratio					
	Trait	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Females	PC1	-0.0453	0.1952	-0.2320	0.8180
	PC2	-0.1722	0.1930	-0.8930	0.3790
	PC3	0.2163	0.1916	1.1290	0.2670
	LD1	-0.0410	0.1952	-0.2100	0.8350
Males	PC1	-0.0762	0.1949	-0.3910	0.6980
	PC2	0.1462	0.1936	0.7550	0.4560
	PC3	0.3261	0.1867	1.7470	0.0903
	LD1	-0.4550	0.1780	-2.5550	**0.0156**
(C) Male biased sex ratio					
	Trait	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Females	PC1	0.0197	0.1989	0.9890	0.3300
	PC2	-0.0502	0.2017	-0.2490	0.8050
	PC3	0.1248	0.2007	0.6220	0.5390
	LD1	-0.2514	0.1969	-1.2760	0.2110
Males	PC1	0.0760	0.201	0.377	0.708
	PC2	0.0091	0.2019	0.0450	0.9640
	PC3	0.2289	0.1978	1.1570	0.2560
	LD1	-0.4047	0.1888	-2.1440	**0.0397**

Table 5b.5 Summary of linear models for sexually concordant fitness index at the three sex ratios with male and female PC1, PC2, PC3 and LD1. P values less than 0.05 are shown in bold.

Linear models with sexual dimorphism as explanatory variable				
(A) Antagonism index and sexual dimorphism				
Sex Ratio	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Female Biased	-0.1807	0.1796	-1.0060	0.3220
Equal	0.0293	0.1565	0.1870	0.8530
Male Biased	0.1329	0.1461	0.9090	0.3700
(B) Sexually concordant fitness component and sexual dimorphism				
Sex Ratio	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Female Biased	-0.1807	0.1796	-1.0060	0.3220
Equal	-0.4171	0.1817	-2.2950	**0.0284**
Male Biased	-0.1545	0.2008	-0.7700	0.4470
(C) Fecundity of baseline females and sexual dimorphism				
Sex Ratio	**Estimate**	**Std. Error**	**t value**	**Pr(>|t|)**
Female Biased	0.2549	0.1664	1.5320	0.1350
Equal	-0.1263	0.1777	-0.7110	0.4830
Male Biased	0.1329	0.1461	0.9090	0.3700

Table 5b.6 Summary of linear models for (A) antagonism index, (B) sexually concordant fitness index, and (C) fecundity of baseline females held with focal males at different sex ratios with sexual dimorphism along LD1 as the explanatory variable. P values less than 0.05 are shown in bold.

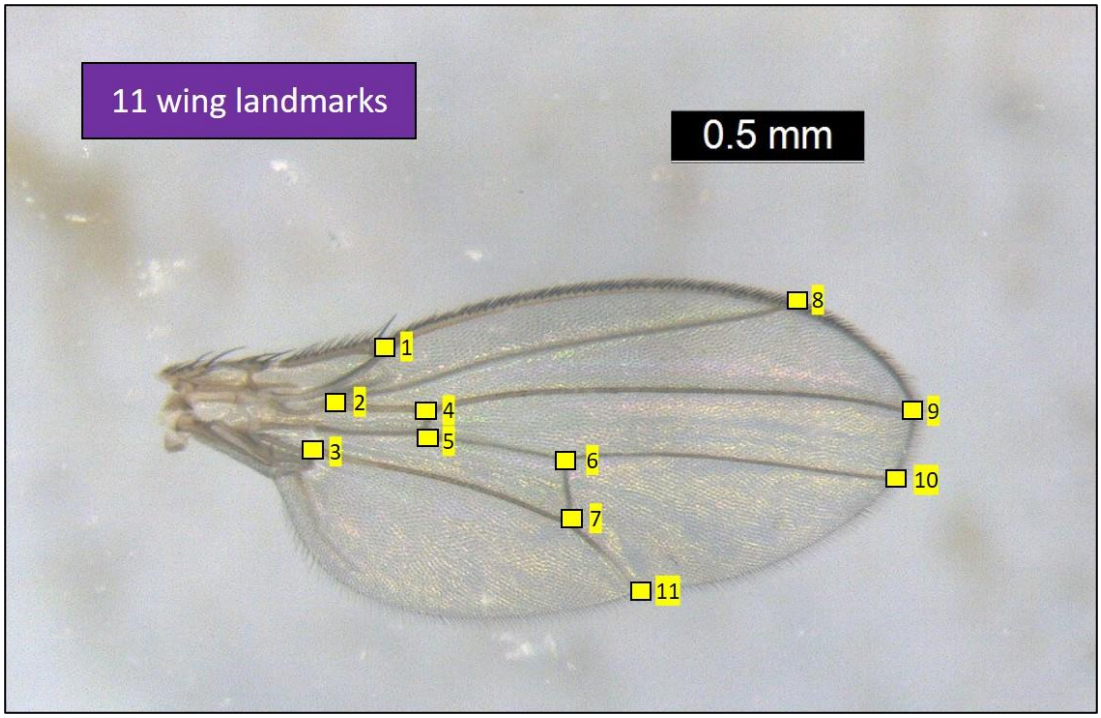

Figure 5b.1 The positions of the 11 different landmarks on the wing surface used for analysis

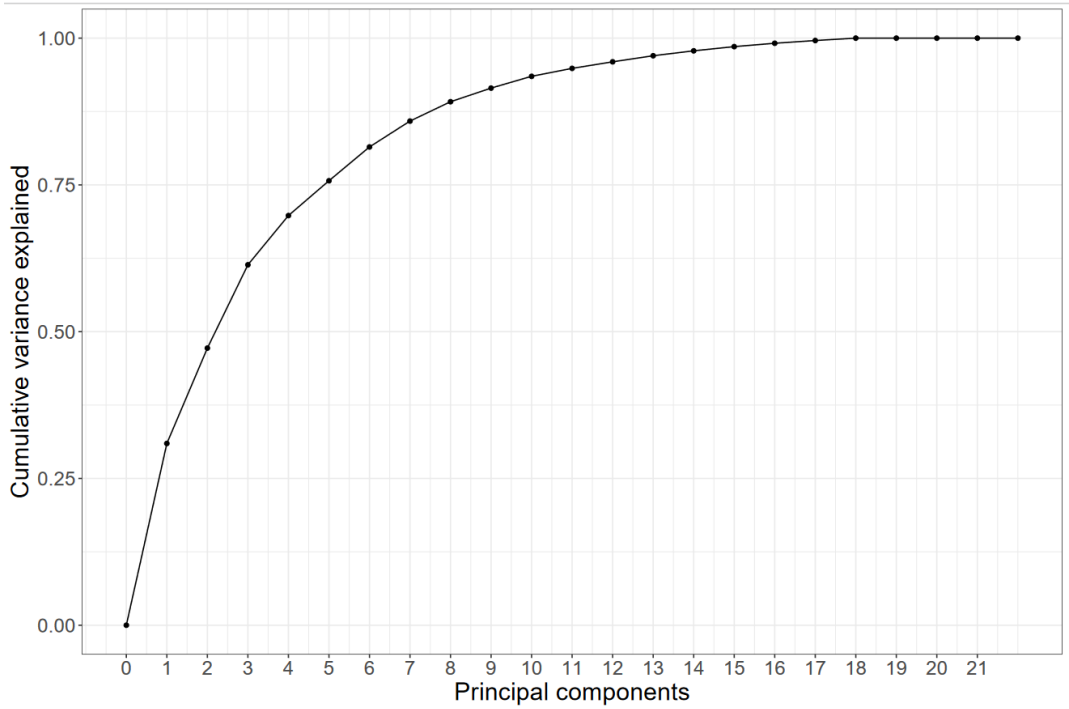

Figure 5b.2 The cumulative variance explained by the principal components for wing shape

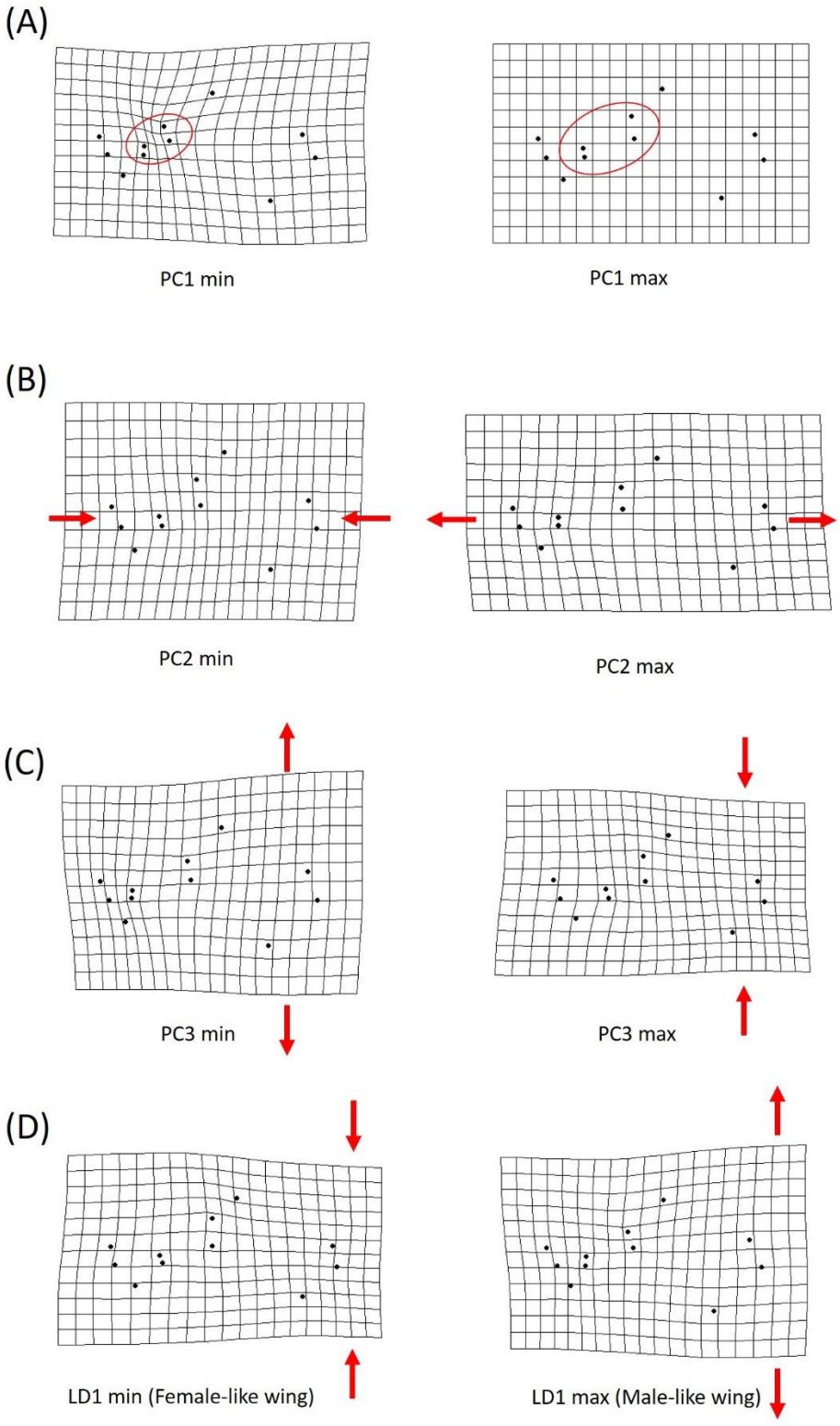

Figure 5b.3 Twice-exaggerated landmark profiles of wings with the least and the highest scores for (A) PC1, (B) PC2, (C) PC3, and (D) LD1. Red circles and arrows highlight the most visible changes in wing shape along the respective axes.

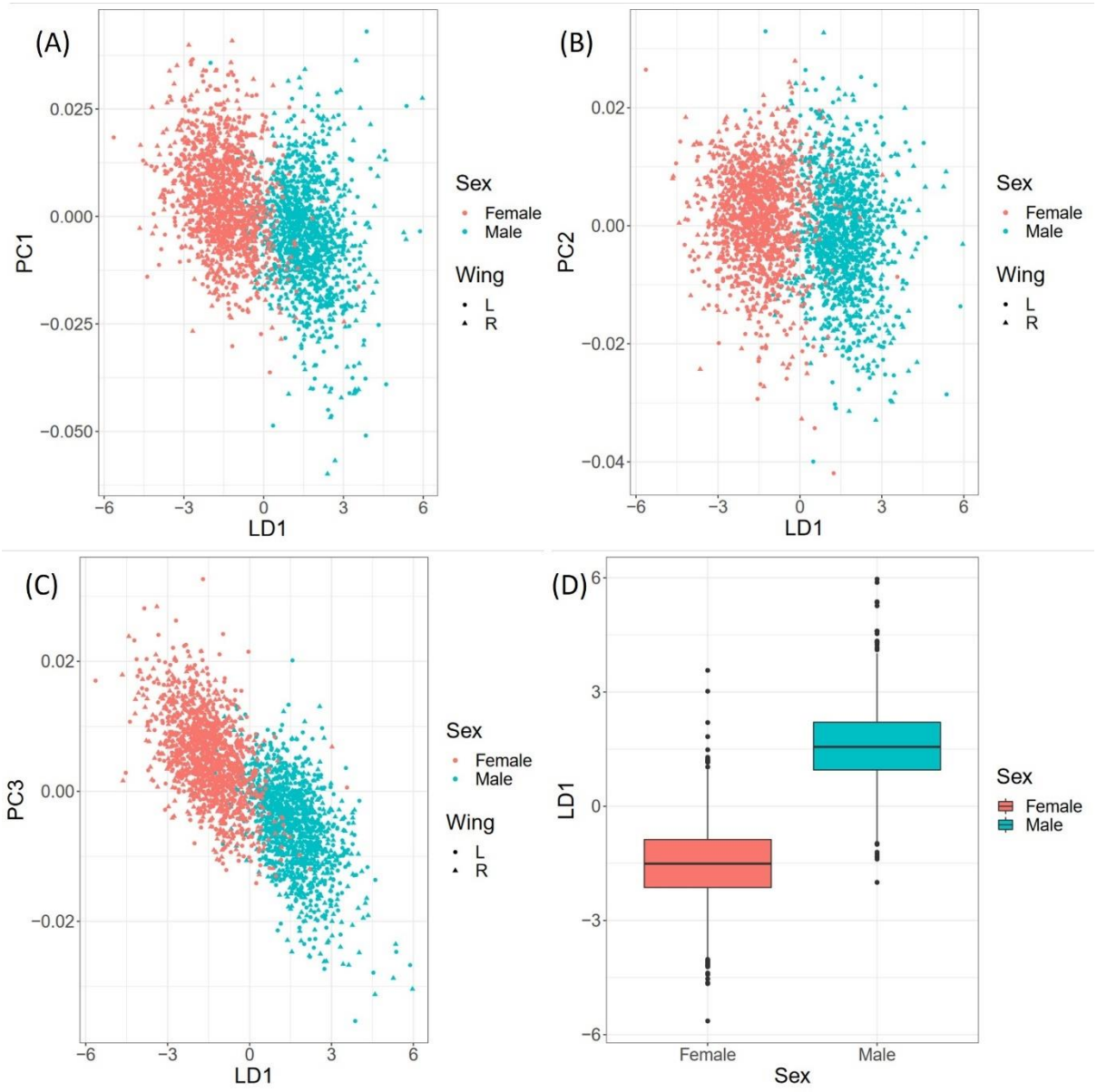

Figure 5b.4 LD1 scores (for sex) plotted for each wing in the data set against (A) PC1, (B) PC2, and (C) PC3. (D) Boxplot depicting sexual dimorphism for wing shape along LD1 axis for sex.

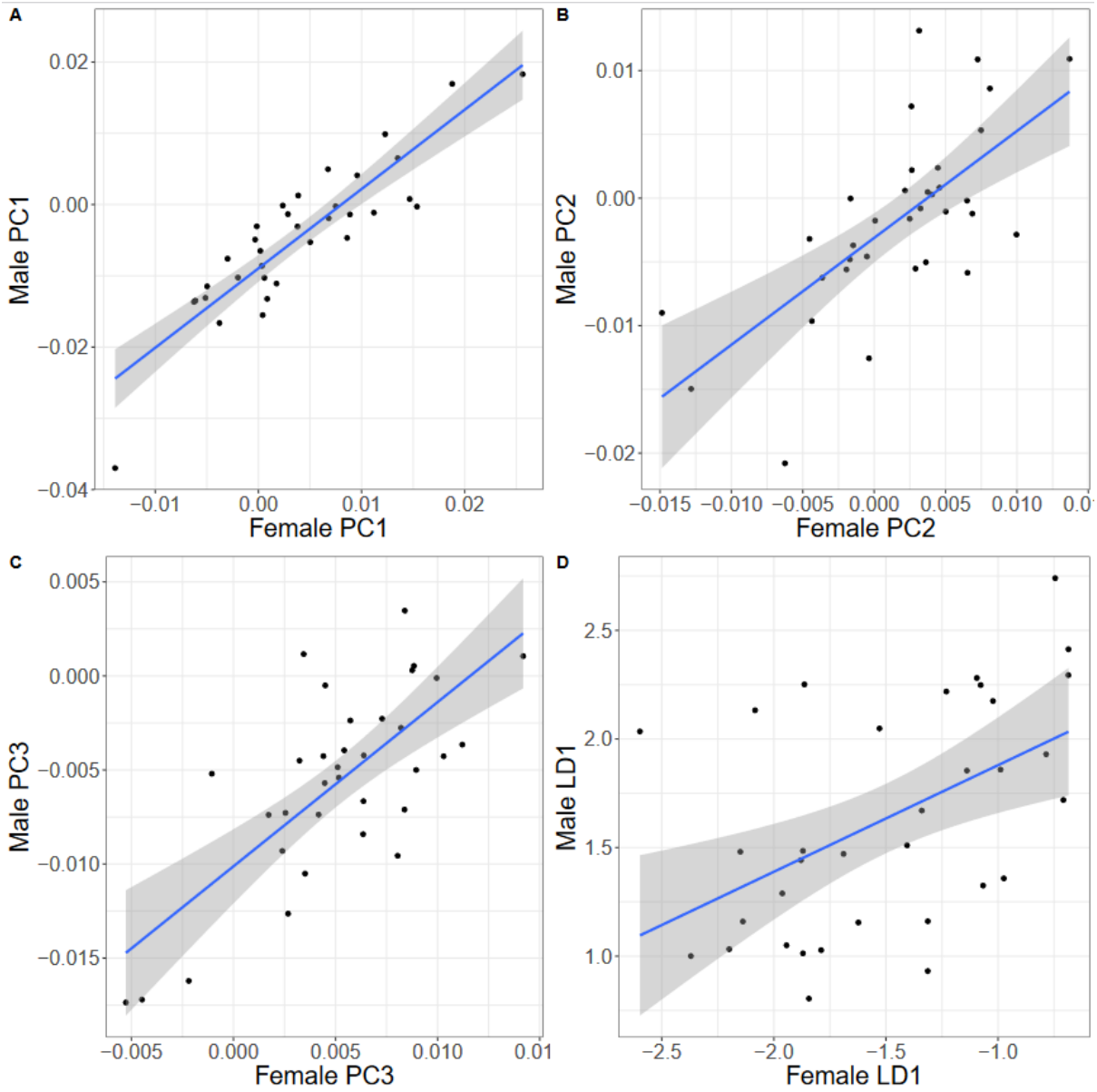

Figure 5b.5 Intersexual genetic correlation for (A) PC1, (B) PC2, (C) PC3, (D) LD1

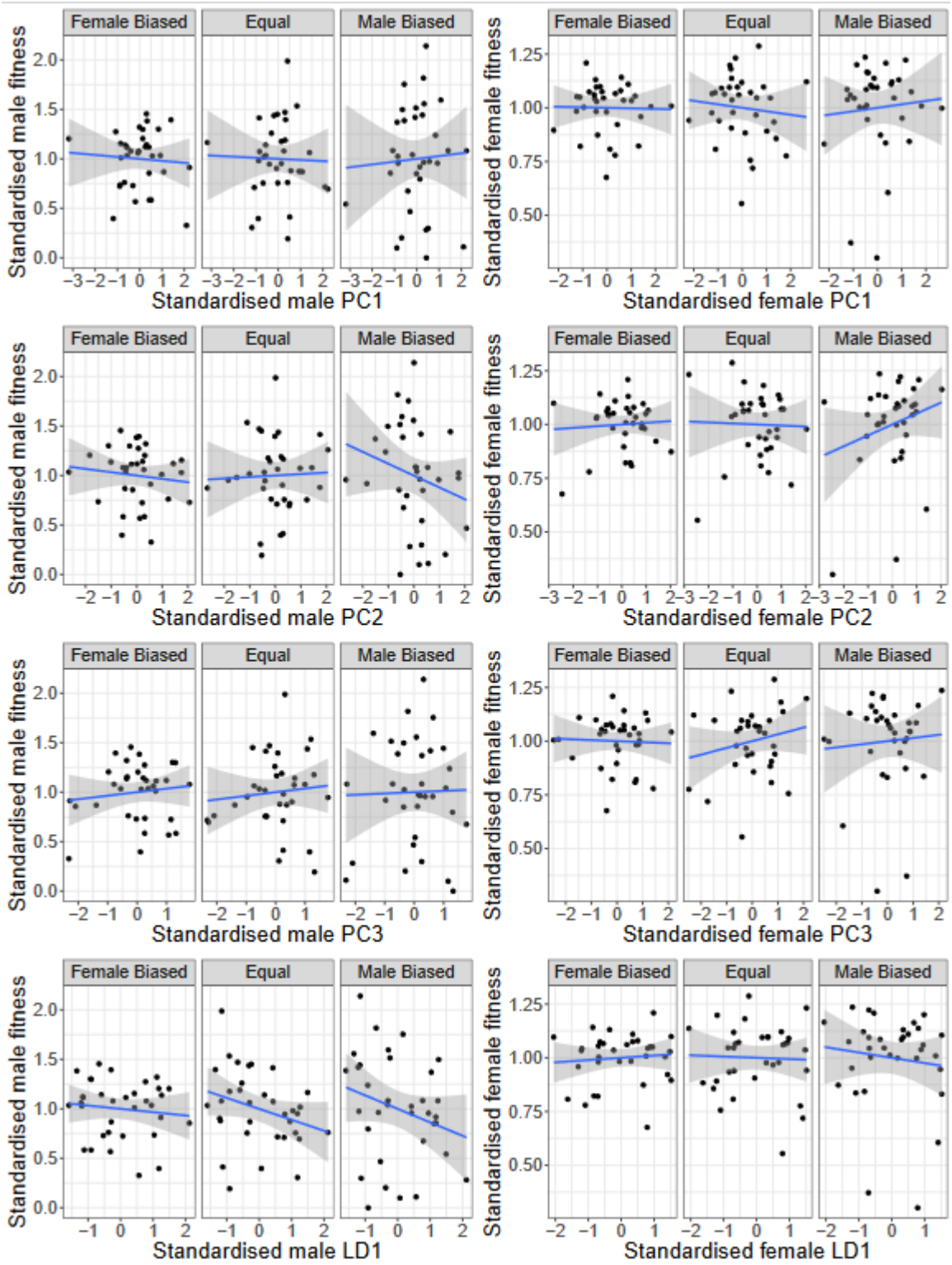

Figure 5b.6 Genetic correlations between relative male and female fitness at the three sex ratios and PC1, PC2, PC3 or LD1 for wing shape. The column on the left represents correlations for males, while the column on the right represents correlations for females. Different rows represent correlations with relative fitness separately for PC1, PC2, PC3 and LD1.

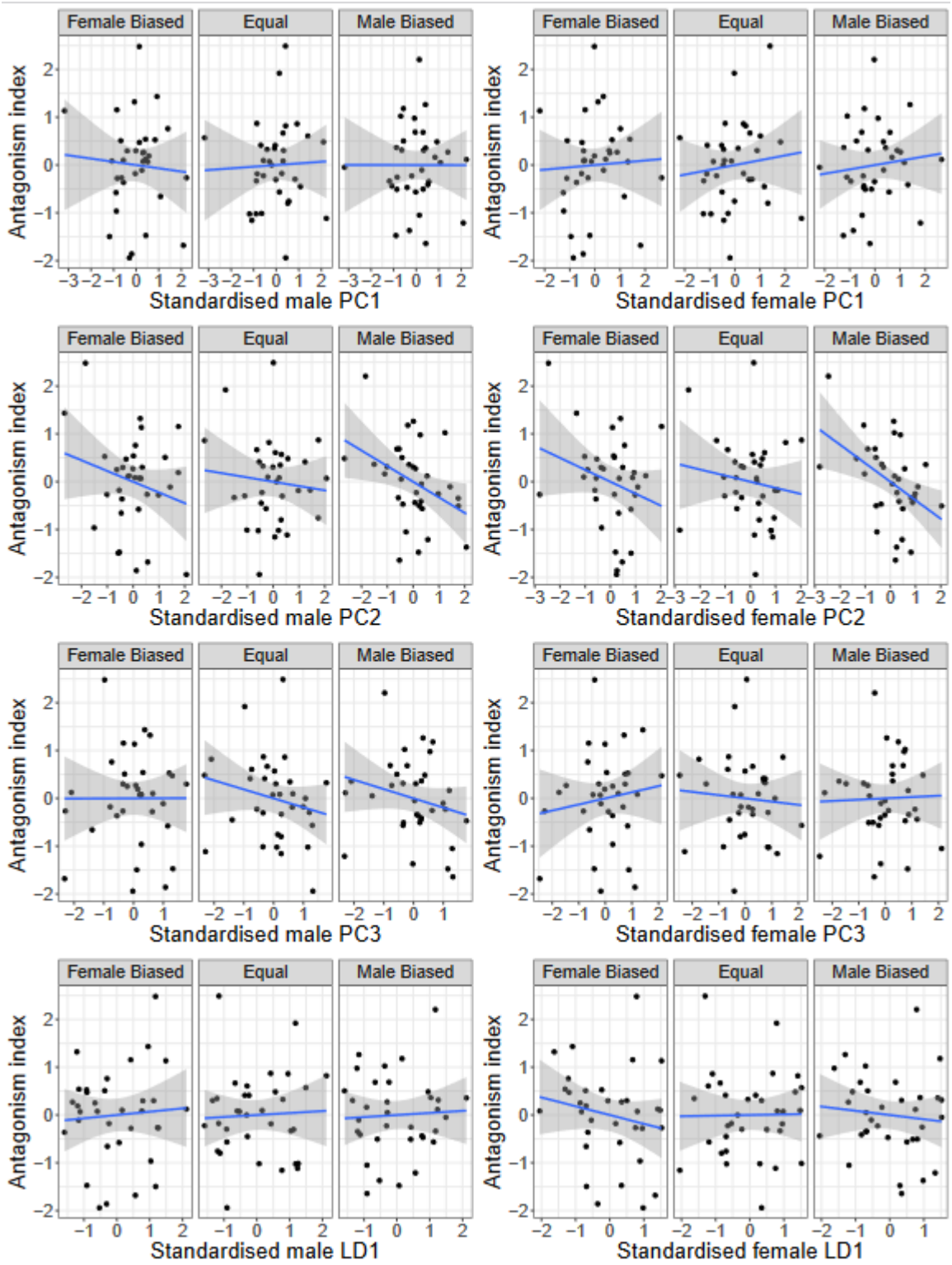

Figure 5b.7 Genetic correlations between antagonism index and male and female PC1, PC2, PC3 or LD1 for wing shape. The column on the left represents correlations for males, while the column on the right represents correlations for females. Different rows represent correlations with relative fitness separately for PC1, PC2, PC3 and LD1.

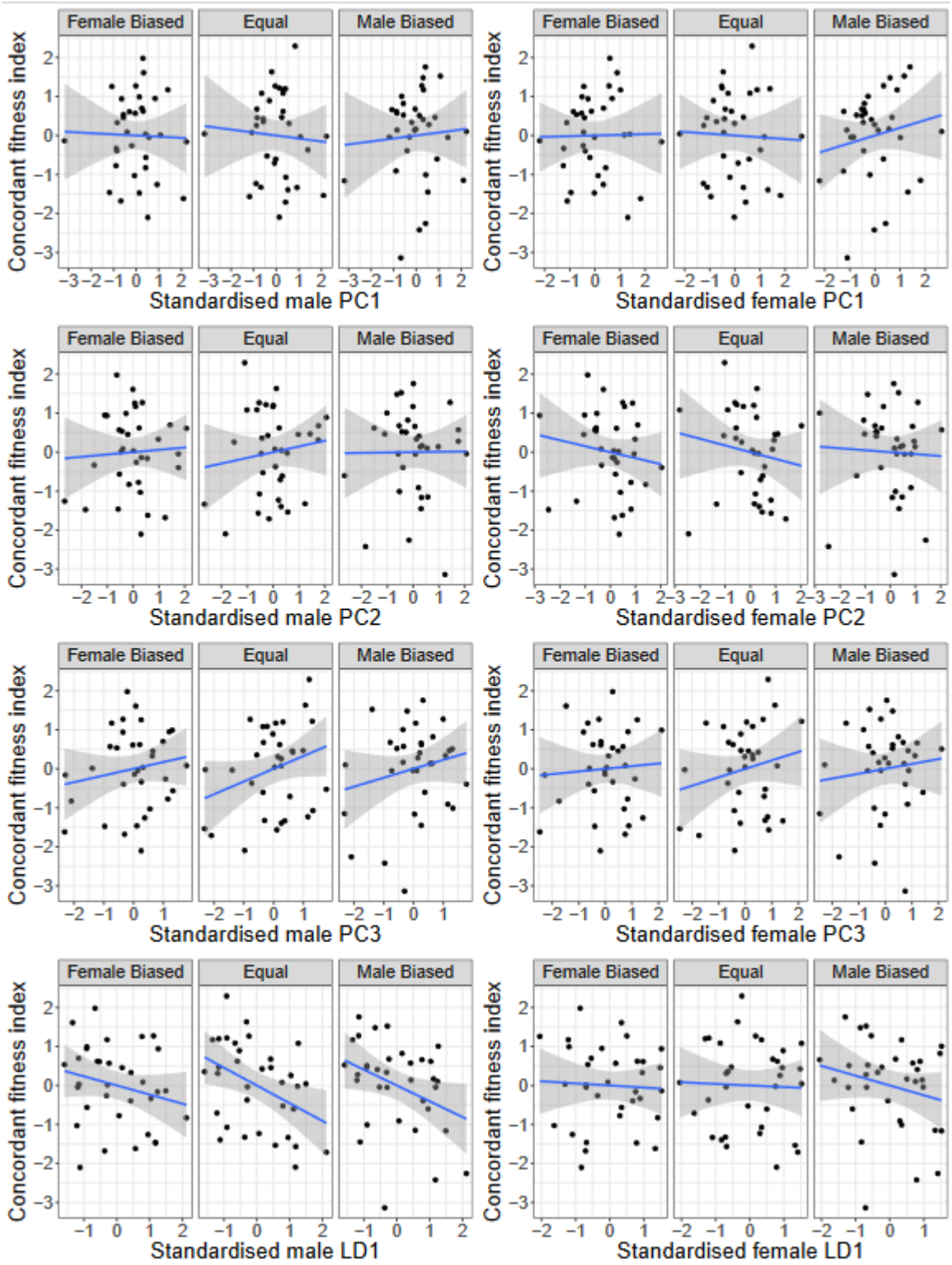

Figure 5b.8 Genetic correlations between sexually concordant fitness index and male and female PC1, PC2, PC3 or LD1 for wing shape. The column on the left represents correlations for males, while the column on the right represents correlations for females. Different rows represent correlations with relative fitness separately for PC1, PC2, PC3 and LD1.

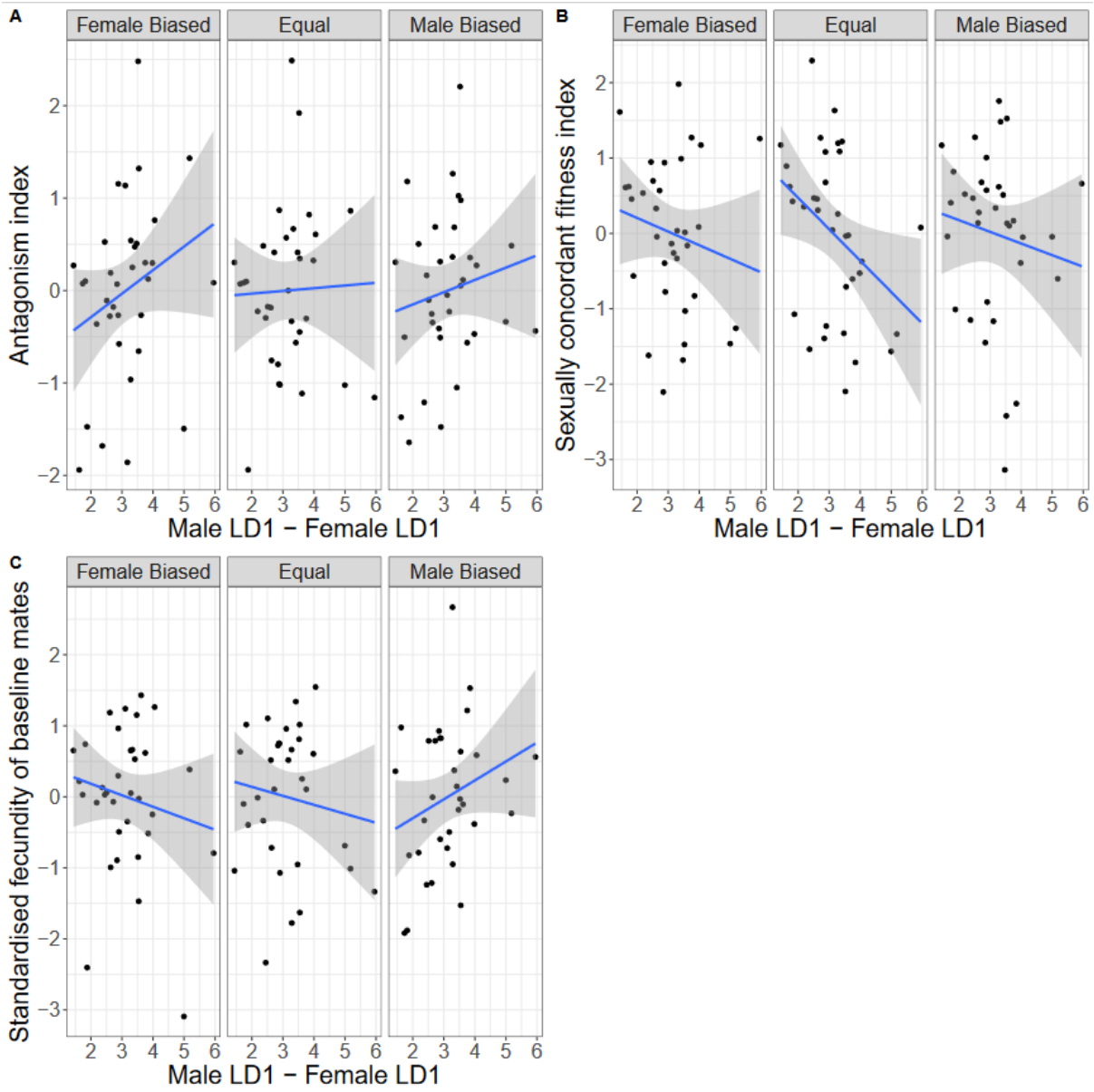

Figure 5b.9 Genetic correlations between sexual dimorphism along LD1 and (A) antagonism index, (B) concordant fitness index, and (C) fecundity of baseline females held with focal males at the three sex ratios

Chapter 6

A two-locus population genetic model for the resolution of Intralocus Sexual Conflict via modifier alleles bringing about sex-biased gene expression

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INTRODUCTION

Intralocus Sexual Conflict (IaSC) ensues when there are distinct sex-specific fitness optima for traits with a common underlying genetic basis in males and females (Bonduriansky and Chenoweth 2009). IaSC can cause populations to remain stuck in a maladapted equilibrium, where neither sex is at their respective fitness maximum. This can exert substantial costs on the average fitness of the population as a whole, and can even lead to an increased risk of extinction (Lande 1980). Numerous empirical studies, particularly over the last two decades, have shown that patterns consistent with IaSC are quite widespread (Berger et al. 2016; Barson et al. 2015; Chippindale et al. 2001; Delph et al. 2011; Eyer et al. 2019; Stulp et al. 2012).

While empirical research on IaSC, and the term "IaSC" itself, are barely a few decades old, the underlying mathematical logic has been the subject of a large number of theoretical studies over the course of the last 70 years. Owen (1953) was the first to extend the standard population genetics framework to incorporate differential selection in the sexes. Owen (1953) showed that a single locus experiencing sex-specific selection in a diploid population can exhibit as many as three different non-trivial equilibria. A number of studies have built upon Owen's (1953) framework to identify conditions that facilitate the invasion of alleles with sex-specific fitness effects (Parsons 1961) and compare sex-specific viability and fertility selection (Bodmer 1965). Sexually antagonistic (SA) selection is a special case of sex-specific selection, where selection coefficients in males and females have opposite signs. Several studies have subsequently evaluated the conditions under which SA alleles can invade a population (Haldane 1962) and be maintained in a stable polymorphism (Haldane 1962; Kidwell et al. 1977). Some studies have compared the efficacy of X chromosomes and autosomes at maintaining SA polymorphisms (Connallon and Clark 2012; Curtsinger 1980; Fry 2010; Pamilo 1979; Patten and Haig 2009; Rice 1984).

While SA selection coupled with the fact that the sexes largely share the same gene pool can constrain adaptation in males and females, there has been a longstanding consensus that SA or at least sex-specific selection is a necessary precondition for the evolution of sexual dimorphism (Andersson 1994; Darwin 1871; Trivers 1972). Using a quantitative genetic approach, Lande (1980) showed that sex-specific natural and/or sexual selection can lead to the evolution of sexual dimorphism, provided there exists additive genetic variation for sexual dimorphism in the population. However, Lande's (1980) quantitative genetic approach did not address the biological mechanism underlying the additive genetic variation for sexual dimorphism. A number of population genetic studies investigating the resolution of IaSC have attempted to fill this gap. These studies have invoked several different biological mechanisms including gene duplication (Connallon and Clark 2011), genomic imprinting (Day and Bonduriansky 2004), sex-specific dominance (Spencer and Priest 2016), and modifier alleles bringing about sex-biased gene expression to model the resolution of IaSC (Connallon and Clark 2010), ultimately leading to sexual dimorphism.

In a seminal study linking gene expression and fitness, Connallon and Clark (2010) adapted a two-locus, diploid population genetics model to a number of different scenarios describing sex-specific and sexually antagonistic selection including antagonistic pleiotropy and SA selection. They were able to show that most values of parameters that allow a SA polymorphism also favour the invasion of a modifier allele bringing about sex-biased gene expression. Furthermore, in a result particularly important in the context of the non-random distribution of SA loci, they also showed that the conditions for expression divergence between the sexes are more stringent on autosomes relative to the X chromosome.

Connallon and Clark's (2010) model clarified considerable confusion stemming from the variation in the fitness schemes employed by theoretical studies in the past and provided a robust theoretical framework that attempted to explain the idiosyncrasies in sex-specific gene expression data (summarised by Dean and Mank (2014) and Jaquiéry et al. (2013)). However, the model’s prediction regarding the ease with which sex-specific genetic architectures can evolve is at odds with the data at the phenotypic level. In spite of pervasive sex-specific (and even sexually antagonistic) selection (Cox and Calsbeek 2009; Singh and Punzalan 2018), strong intersexual genetic correlations persist for a large number of traits (Poissant et al. 2010). One of the reasons for this dissonance could be a simplifying assumption made by Connallon and Clark (2010). In their model, they assumed when

divergence in expression proceeded via exaggeration through males, the modifier allele only affected the expression of the male beneficial allele in females, but not the expression of the female beneficial allele in females. On the other hand, when divergence proceeded via exaggeration in females, the modifier only affected the expression of only the female beneficial allele in males. It remains to be investigated whether this is a reasonable assumption. Sex-specific modifier alleles are expected to be transcription factors that are linked to the sex-determination pathway. While they limit the expression of an allele at a certain locus in one of the sexes, it is unlikely that they have absolutely no effect on another allelic variant at the same locus. There is considerable evidence for allele-specific gene regulation. However, the assumption made by Connallon and Clark (2010) describes a highly idealised scenario where the modifier entirely shuts down the expression of only one of the alleles in one of the sexes, while leaving the other allele completely unaffected. More realistically modifiers would alter the expression of both alleles, but to different degrees (see Discussion for a plausible model for the underlying molecular biology). It is, therefore, important to investigate whether the resolution of IaSC is indeed as easily attained under these slightly more realistic conditions as it is under the conditions assumed by Connallon and Clark (2010).

In this study, I altered a variant of Connallon and Clark's (2010) model for the evolution of sex-biased gene expression at a SA. I specifically allowed the modifier allele to modulate the expression of *both* alleles at the SA locus in one of the sexes, and explored the relationship between the effect of the modifier allele on the expression of the two alleles and the likelihood of the resolution of IaSC.

MODEL

In this chapter I present a two-locus model for the resolution of IaSC via modifiers that bring about sex biased gene expression. For simplicity, this model only considers selection acting in the haploid stage. Therefore, the results are relevant to organisms with a prominent gametophytic stage. In this system a diploid sporophyte produces haploid spores after meiosis. The sporophytic stage is minor and not under strong selection. The spores develop into haploid gametophytes. The gametophytic phase is important and is under viability selection. After differential viability, the gametophytes produce gametes. There is random mating among haploid gametes to produce diploid zygotes that develop into sporophytes.

I consider two loci. Locus A is the sexually antagonistic locus, with A1 (A2) being the male (female) beneficial allele. Locus M is the modifier locus, where M2 is a null allele, while M1 is the modifier allele that modulates expression patterns at locus A, but only in females. Since this system is symmetric with respect to males and females, the model for modification of expression in males is identical to this model with male and female labels swapped. If the modifier allele M1 is absent, the fitness scheme for the one-locus system is given in Table 6.1. Selection coefficients in males and females are represented by a and b respectively. It can then be shown that this system exhibits a stable polymorphism at which the frequency of A1 is given by $p_A^* = \frac{1}{2} + \frac{1}{2}(\frac{1}{b} - \frac{1}{a})$ provided the following condition is met: $-1 < \frac{1}{b} - \frac{1}{a} < 1$. This condition corresponds to the polymorphic equilibrium described by (Kidwell et al. 1977b) for the case where fitnesses are additive, albeit with a slightly different parameterisation. In the two-locus system, let x11, x12, x21 and x22 represent the frequencies of haplotypes A1M1, A1M2, A2M1 and A2M2. Let r represent the recombination rate between locus M and locus A. The general iterative equations for a two-locus system with haploid viability selection are given in the Appendix of this chapter and have been adapted from Rice (2004).

The fitness scheme for the two-locus model is shown in Table 6.2. M2 modulates the expression pattern at the SA locus A. The effect of M2 on the fitness of A1 and A2 alleles in females is controlled by two parameters, k1 and k2 respectively. Both k1 and k2 are allowed to vary between 0 and 1. If k1 is large, the modifier "protects" the females from the deleterious effects of the male beneficial allele; at k1 = 1, the male beneficial allele acts exactly like the female beneficial allele in females. If k2 is 1, the modifier does not affect the fitness of the female-beneficial allele in females at all. If k2<1, the modifier allele reduces the benefits of A2 in females. Therefore, modifier alleles for which both k1 and k2 large should be best-suited for the resolution of IaSC.

Linear stability analysis

When M1 is absent, the equilibrium frequencies of the haplotypes are given by the following expressions:

x11* = 0

x21* = 0

$x12^* = \frac{1}{2} + \frac{1}{2}(\frac{1}{b} - \frac{1}{a})$

To investigate the parameter space for regions where the modifier allele M1 could invade the population, I examined the stability of the fixed point described above by performing linear stability analysis. Using Wolfram Mathematica, I computed the Jacobian matrix for the system of iterative equations, as well as the corresponding eigenvalues. I evaluated the modulus of the eigenvalues at the fixed point. I then numerically classified the parameter space into regions where the modulus of all the eigenvalues was less than 1, where the fixed point was stable, and regions for which the modules of at least one of the eigenvalues exceeded 1, which corresponded to the fixed point not being stable. Note that the fixed-point losing stability is an essential precondition for the invasion of M1. For each pair of values of k1 and k2, I calculated the proportion of parameter space in a and r (keeping b constant at either 0.2 or 0.02) for which the fixed point was stable. In these calculations I varied r between 0 and 0.5, and a between b/(1+b) and b/(1-b) (which is the parameter space where SA selection maintains a polymorphism at locus A in absence of M1). Therefore, this analysis investigates efficacy of various kinds of modifier alleles at resolving IaSC, given a sexually antagonistic polymorphism is present to begin with. Note that a greater probability of stability corresponds to a reduced probability that the modifier resolves IaSC.

Equilibrium behaviour and initial conditions

I complemented the linear stability analysis by iterating the system of recursive relations that describe the system to investigate equilibrium behaviour as well as certain properties of initial conditions for various values of parameters. First, I plotted the frequency of allele A1, and allele M1 after 3000 generations for various values of k1, k2, and a, while keeping b and r constant at 0.2 and 0.1 respectively. Next, I calculated the minimum frequency of the modifier allele M1 that needs to be introduced to a population polymorphic at the fixed point, for the A1M1 haplotype to go to fixation, separately for various pairs of values of k1 and k2. As before, b and r were kept constant at 0.2 and 0.1 respectively. In order to calculate the minimum frequency of M1 that led to the fixation of A1M1, I started with M1 = 0.01, and then increased M1 in steps of 0.01. The initial linkage disequilibrium between locus A and locus M was set to 0. At each step, I ran the model for 3000 generations and noted the equilibrium frequency of A1M1. I recorded the minimum initial frequency of M1 for which A1M1 went to fixation.

RESULTS

In absence of the modifier allele M1, the condition for the maintenance of a polymorphism at locus A is shown in Figure 6.1. The conditions for the maintenance of a stable polymorphism at locus A are quite restrictive when selection is weak. Using linear stability analysis, I investigated the potential of various kinds of modifier alleles (i.e., modifier alleles with different values of k1 and k2). Assuming condition depicted in Figure 6.1 was satisfied, and IaSC was present, for each pair of values of k1 and k2, I calculated the proportion of parameter space in a and r (keeping b constant) for which the fixed point was stable. The results for strong selection (b = 0.2) and weak selection (0.02) are depicted in Figure 6.2a and Figure 6.2b respectively. Warmer colours indicate a greater proportion of parameter space for which the fixed point was stable, and therefore IaSC was left unresolved. Cooler colours depict regions of the parameter space, where the modifier allele M1 could invade the population, bringing about sex-biased expression. Whenever k2 was 1, i.e., the modifier allele did not affect the expression of the female beneficial allele in females, the fixed point was never stable as long as k1 was greater than 0. However, when k2 was less than 1, the fixed point was stable for some values of a and r, suggesting that resolution of IaSC was not automatically guaranteed. When k1 and k2 both were large, the proportion of parameter space in a and r permissible for the resolution of IaSC was also large. On the other hand, small values of k1 and k2 corresponded to regions where the fixed point was largely stable, and IaSC was not resolved. An interesting aspect of my results was that regions indicated by cooler colours (i.e., regions where IaSC was more readily resolved) were larger when selection was stronger (b = 0.2, Figure 6.2a) than when selection was weaker (b = 0.02, Figure 6.2b). However, it must be noted that this analysis was restricted to the parameter space where a polymorphic equilibrium would be present at locus A in absence of M1 (i.e., the region bounded by the curves in Figure 6.1). Figure 6.3 depicts, for various pairs of values of k1 and k2, the regions of parameter space in a and r (keeping b constant at 0.2) for which the fixed point was stable (yellow) and IaSC was not resolved and regions where the fixed-point lost stability (black), paving the way for the invasion of the modifier allele M1. The modifier that does not protect females from the deleterious effects of the male beneficial allele A1 (k1 = 0), cannot invade the population (Figure 6.3a). On the other hand, the modifier that does not affect the expression of A2 in females (k2 = 1), but protects females from the deleterious effects of A1 (k1>0) is guaranteed to invade the population (Figure 6.3b). When both k1 and k2 are between 0 and

1, larger values of k1 and k2 are more conducive for the invasion of the modifier allele, as indicated by the larger black region in the top right corner of Figure 6.3c. Furthermore, increasing a (while keeping b constant) favours the invasion of M1, while larger values of the recombination rate r impede the invasion of M1, particularly at smaller values of a.

My analysis of the equilibrium behaviour and initial conditions was consistent with the linear stability analysis. A1M1 went to fixation for the smallest value of the selection coefficient in males, a, for the modifier allele with k2 = 1 (yellow curves in Figure 6.4 and Figure 6.5). On the other hand, when k1 was 0, the strength of selection in males (i.e., a) had to be the highest for A1 to go to fixation, while M1 could never go to fixation (black curves in Figure 6.4 and Figure 6.5). When k1 and k2 were both set at 0.8, A1 and M1 got fixed at low values of a (green curves in Figure 6.4 and Figure 6.5). When k1 and k2 were both 0.2, it required much larger values of a (red curves in Figure 6.4 and Figure 6.5). The results from the analysis of the initial frequency of M1 mirrored these findings. The modifier allele for which k1 was 0 required an initial frequency of 1 for A1M1 to get fixed (black curve in Figure 6), while the modifier allele for which k2 was 1 required the smallest initial frequency of M1 (yellow curve in Figure 6). Large values of k1 and k2 corresponded to smaller initial frequency of M1 required for the fixation of A1M1 (green curve), while small values of k1 and k2 corresponded to a larger initial frequency of M1 (red curve).

DISCUSSION

One of the most commonly invoked mechanisms for the resolution of IaSC is the evolution of sex-biased gene expression at SA loci through modifiers of gene expression. In a landmark study Connallon and Clark (2010) showed, among other important results, that a modifier reducing the expression of the deleterious allele in one of the sexes could invade the populations. In this chapter, I modified a version of Connallon and Clark's (2010) model by specifically allowing the modifier to modulate the expression of both male beneficial as well as female beneficial alleles in females at the SA locus and examined the efficacy of various kinds of modifier alleles at resolving IaSC. My main findings are as follows:

1. As long as the modifier allele M1 reduces the benefits of A2 to females even slightly, while protecting females from the harmful effects of A1, the resolution of IaSC is not automatically guaranteed.

2. Resolution of IaSC through the fixation of the male beneficial allele A1, along with the modifier allele M1 is favoured by stronger selection in males, but is inhibited by faster recombination rates.

Note that, for the case when dimorphism evolves by divergence through females, driven by modifiers that modulate expression in males, the results are the same as described above, with labels of males and females swapped.

Sex-specific, and even SA, selection is incredibly common in nature (Cox and Calsbeek 2009; Singh and Punzalan 2018). A large number of theoretical studies predict that such selection can favour the evolution of sex-specific genetic architecture resolving IaSC (Connallon and Clark 2010; Connallon and Clark 2011; Day and Bonduriansky 2004; Spencer and Priest 2016). Consistent with theoretical predictions, patterns of sex biased gene expression have been reported in many organisms (Ellegren and Parsch 2007; Grath and Parsch 2016). However, most studies that have investigated sex-biased gene expression have assumed that extant sex bias in gene expression is a relic of past SA selection, without providing any independent evidence (but see Wright et al. (2018)). Some studies have argued that sex-biased gene expression can arise via evolutionary mechanism other than IaSC, and it may not be a reliable indicator of SA selection (Agrawal 2018; Kasimatis et al. 2017; Rowe et al. 2005; Ruzicka et al. 2019). Often, sex bias in gene expression is limited to the gonads, and genes expressed in somatic tissues are rarely expressed in a sex-biased manner (Stewart et al. 2010). It is, therefore, not very surprising that these patterns at the molecular level seldom translate to patterns at the phenotypic level (Dean and Mank 2014). In fact, in spite of strong sex-specific selection (Singh and Punzalan 2018), strong intersexual genetic correlations have persisted (Poissant et al. 2010), preventing sex-specific adaptation. Stewart and Rice (2018) performed artificial sexually antagonistic selection on body size in *Drosophila melanogaster*, which has a strong intersexual genetic correlation for body size. They showed that in spite of strong disruptive selection between the sexes, body size did not evolve in a sex-specific manner for a substantial period of time. Taken together, these findings seem to suggest that intersexual genetic correlations may not be as easily resolved via the evolution of sex-specific genetic architecture, as previously thought.

It is in this context that my finding, suggesting that the resolution of IaSC may not be always guaranteed via modifiers brining about sex-biased gene expression, is relevant.

Crucially, I showed that upon relaxing Connallon and Clark's (2010) assumption that the modifiers only reduce the expression (in one of the sexes) of the deleterious alleles while leaving the beneficial allele unaffected, invasion of modifiers becomes more constrained. To understand why Connallon and Clark's (2010) assumption may reflect a highly idealised scenario, it might be instructive to think of what it translates to in terms of the molecular biology of the system. While there is ample evidence of allele-specific regulation of gene expression (Buckland 2004; Knight 2004; Pastinen 2010), it is not clear whether this is a product of the complete silencing of one allele accompanied by no change to the expression of the other allele. Additionally, in Connallon and Clark's (2010) case the regulator (i.e., the modifier allele) needs to perform such highly extreme allele-specific regulation only in one of the sexes. Imagine that the modifier allele M1 is a transcription factor that controls the expression at locus A only in females by binding to the promoter and preventing transcription. In theory, it is possible that M1 binds to the promoter of A1 with a very good efficiency, but does not bind to the promoter of A2 *at all*. However, a more realistic scenario is one where M1 binds to the promotors of both A1 and A2, but with different binding efficiencies, such that the expression levels of both A1 and A2 are both reduced, but to different degrees. In my model, in some sense, k1 and k2 model the binding efficiencies of M1 to the promoters of A1 and A2, respectively. My results imply that as long as M1 can bind to the promoters of both A1 and A2, as opposed to only the promoter of A1, conditions favouring the invasion of M1, and therefore, the resolution of IaSC, become much more difficult. This argument can also be extended to scenarios when expression regulation happens by chromatin remodelling or by micro RNAs.

The second interesting feature of my results is the role played the recombination rate. My results suggest that lose linkage between the modifier locus and the SA locus, i.e., greater recombination rates, inhibits the invasion of the modifier allele, particularly when the strength of selection in males is weak. In my model, M1 can only got to fixation in linkage with A1. Therefore, a competition between selection favouring the A1M1 haplotype and recombination dismantling the favourable haplotype determines the outcome. A corollary of this result is that sex-biased genes should be more common in genomic regions with low rates of recombination compared to regions with high rates of recombination. A straightforward way to test this prediction would be to combine data on the genomic distribution of sex-biased genes with data on the genome-wide variation in recombination rates. Such data, definitely exists for organisms like *Drosophila melanogaster* (Zhang and

Parsch 2005). A logical next step for this study could be to examine the density of sex-biased genes and local recombination rates.

CONCLUSION

My analyses suggest resolution of IaSC via sex-biased gene expression brought about by modifier alleles is only guaranteed if the modifier has some highly restrictive properties; i.e., the modifier reduces the expression of the deleterious allele, without affecting the expression of the beneficial allele. Relaxing this assumption reveals that the resolution of IaSC may be more difficult than previously thought, and may depend in the strength of selection as well as the recombination rates.

Genotype	**A1**	**A2**
Fitnesses in Males	1+a	1
Fitnesses in Females	1	1+b
Frequencies	p_A	q_A

Table 6.1 Fitness scheme for the one-locus model with sexually antagonistic selection. Both a and b are constrained to vary between 0 and 1.

Genotype	**A1M1**	**A1M2**	**A2M1**	**A2M2**
Fitnesses in Males	1+a	1+a	1	1
Fitnesses in Females	1+bk1	1	1+bk2	1+b
Frequencies	x11	x12	x21	x22

Table 6.1 Fitness scheme for the two-locus model with sexually antagonistic selection at locus A coupled with a modifier locus M. a, b, k1 and k2 are constrained to vary between 0 and 1.

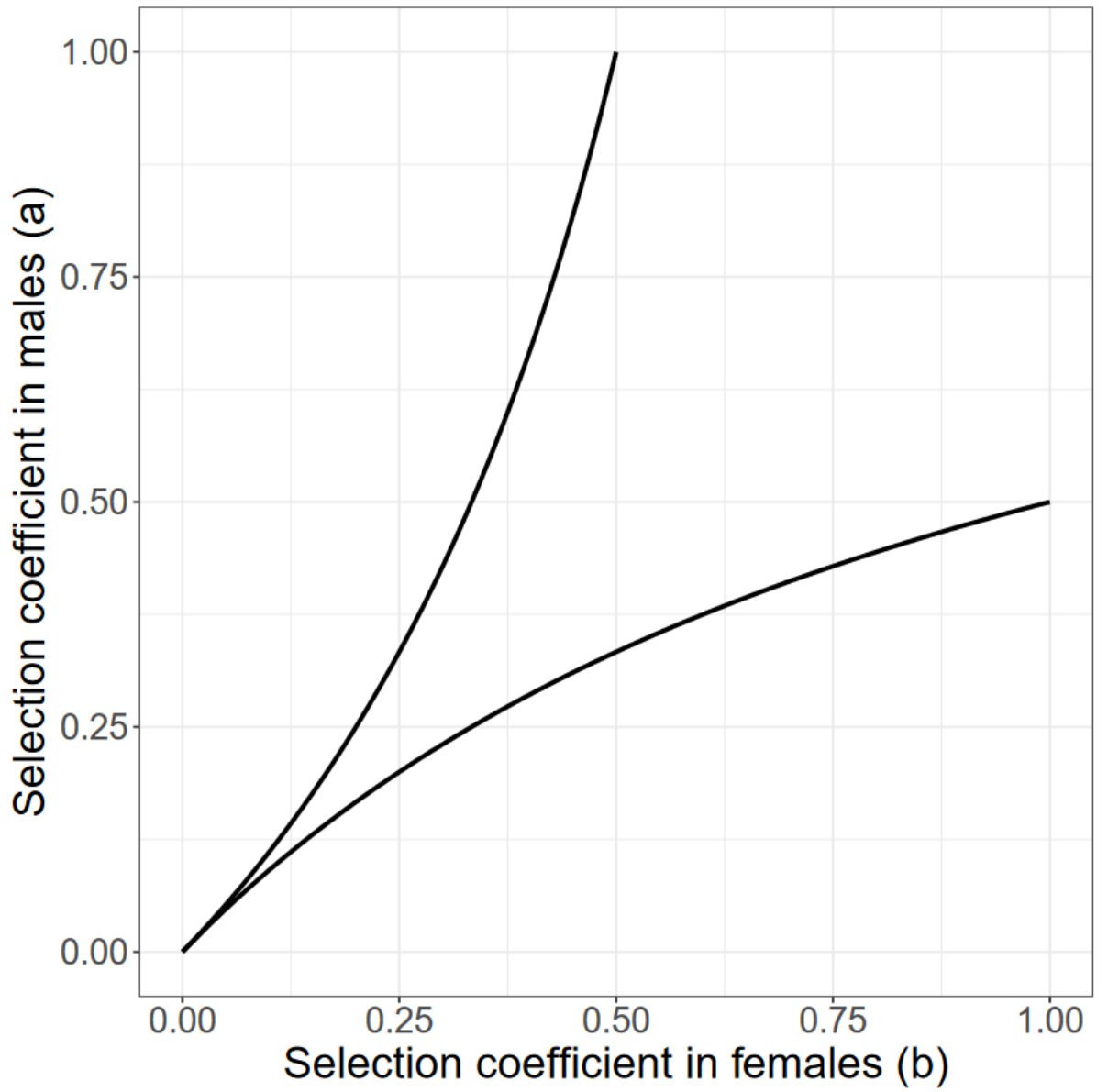

Figure 6.1. The conditions for the maintenance of a stable polymorphism at the sexually antagonistic locus A, when the modifier allele M1 is absent. The region bounded by the two solid curves is the region where a stable polymorphism is permissible.

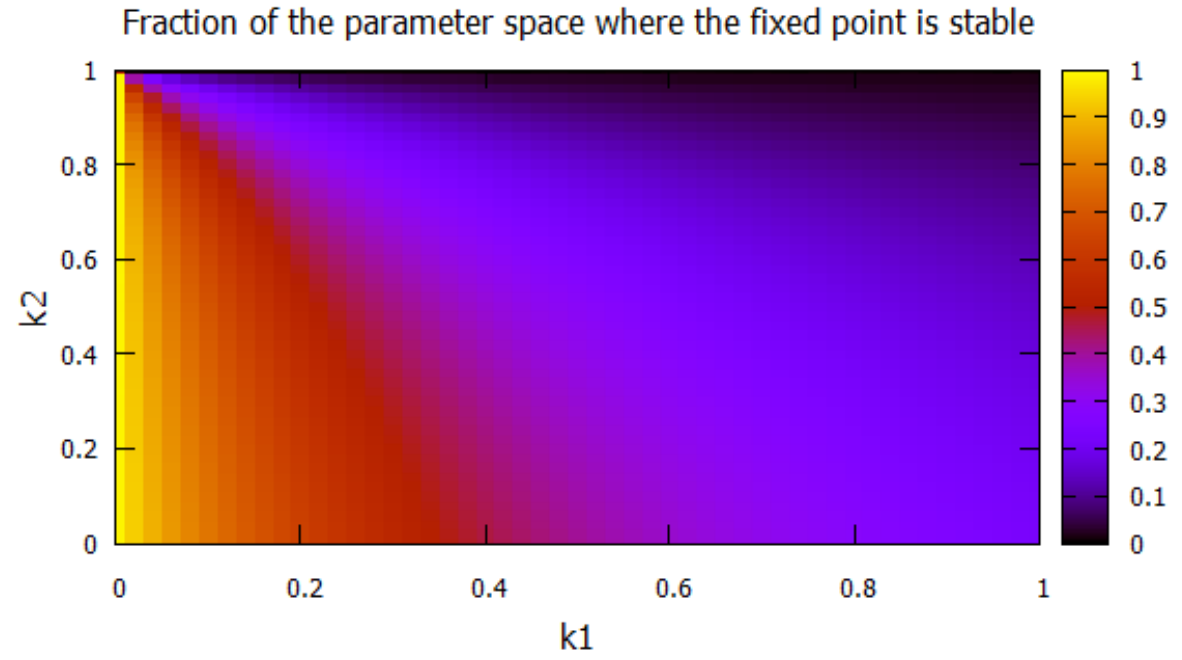

Figure 6.2a. The proportion of parameter space in a and r for which the fixed point was stable under conditions of strong selection (b = 0.2). a was varied between b/(1+b) and b/(1-b), and r between 0 and 0.5.

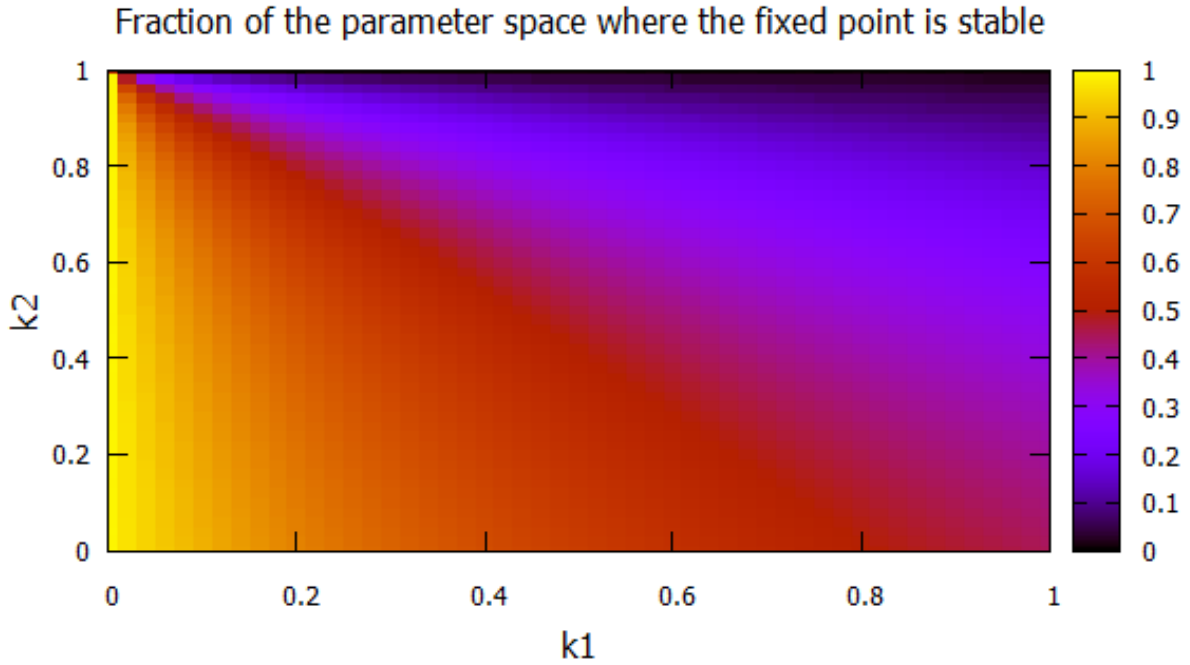

Figure 6.2b. The proportion of parameter space in a and r for which the fixed point was stable under conditions of weak selection (b = 0.02). a varied between b/(1+b) and b/(1-b), and r between 0 and 0.5.

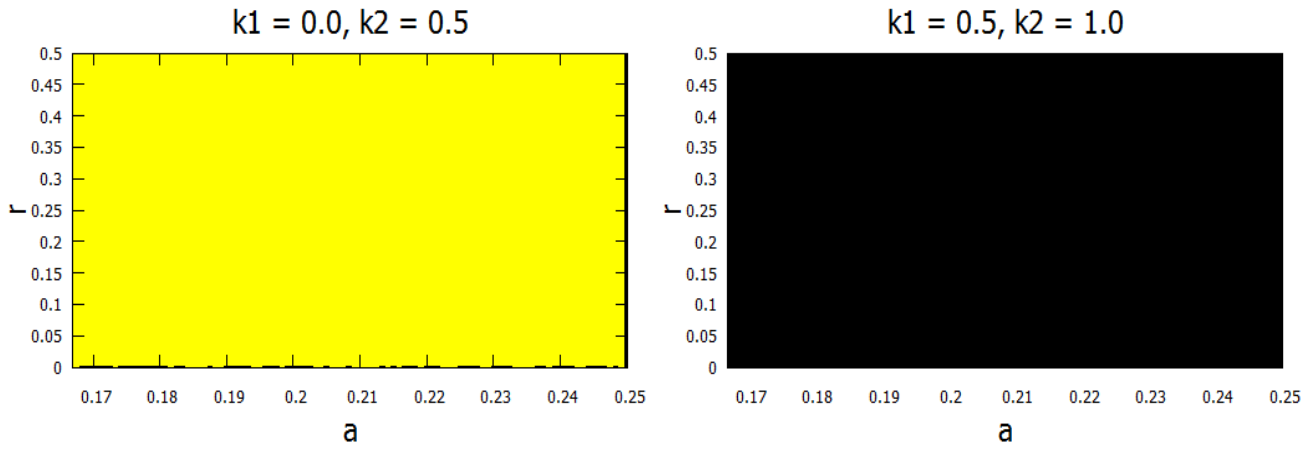

Figure 6.3a. Regions of parameter space in a and r where the fixed point was stable (yellow) or unstable (black) when k1 = 0 and k2 = 0.5 (left), or k1 = 0.5 and k2 = 1 (right) obtained by linear stability analysis. b and r were kept constant at 0.2 and 0.1 respectively.

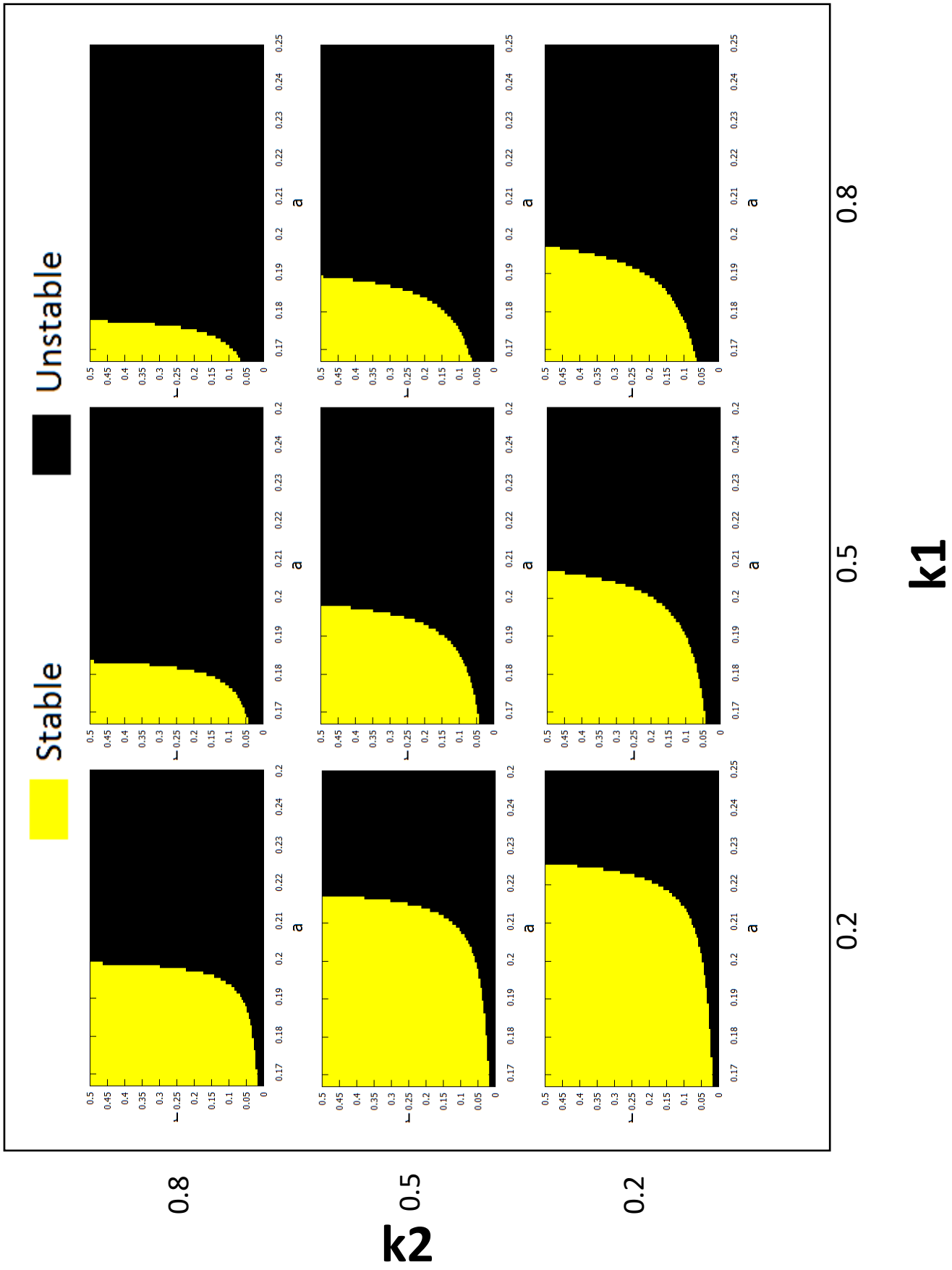

Figure 6.3b. Regions of parameter space in a and r where the fixed point was stable (yellow) or unstable (black) for various values of k1 and k2, obtained by linear stability analysis. b and r were kept constant at 0.2 and 0.1 respectively.

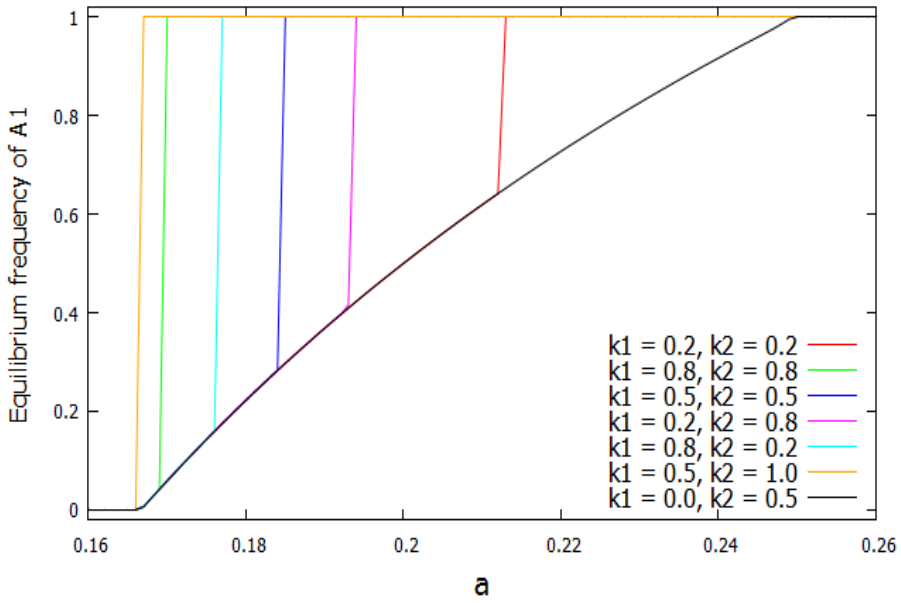

Figure 6.4. The equilibrium frequency of A1 after 3000 generations for various pairs of values of k1 and k2 (indicated by different colours) as well as a. b and r were kept constant at 0.2 and 0.1 respectively. The initial frequency of A1, M1 and the linkage disequilirium between locus A and locus M were set at $\frac{1}{2}+\frac{1}{2}(\frac{1}{b}-\frac{1}{a})$, 0.01, and 0.

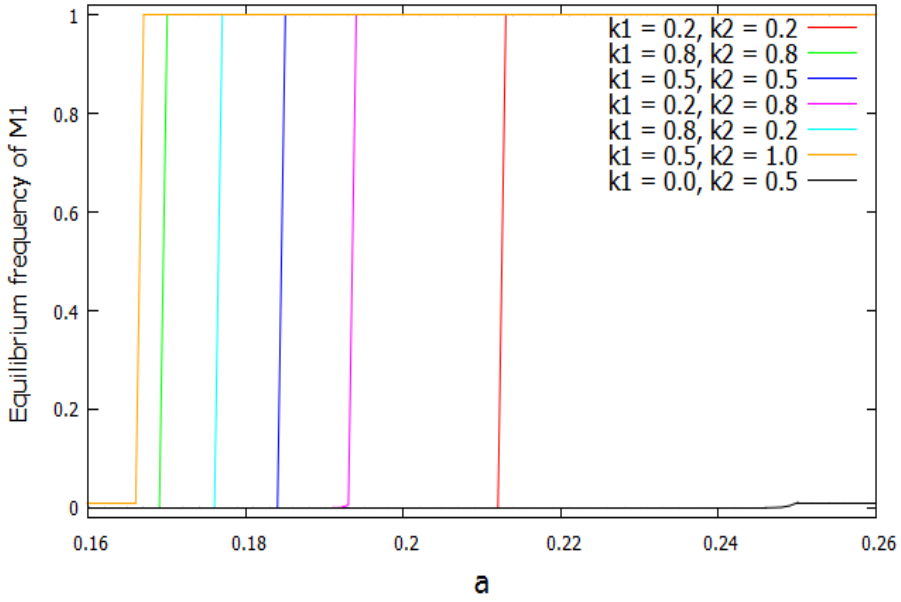

Figure 6.5. The equilibrium frequency of M1 after 3000 generations for various pairs of values of k1 and k2 (indicated by different colours) as well as a. b and r were kept constant at 0.2 and 0.1 respectively. The initial frequency of A1, M1 and the linkage disequilirium between locus A and locus M were set at $\frac{1}{2}+\frac{1}{2}(\frac{1}{b}-\frac{1}{a})$, 0.01, and 0.

APPENDIX

The system of iterative equations for the fitness scheme described in Table 2:

x11’=x11×((1+a)/(x11×(1+a)+x12×(1+a) + x21 + (1-x11-x12-x21)))×x11×((1+k1×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b))) + x11×((1+a)/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x12×(1/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + x11×((1+a)/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x21×((1+k2×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + x11×((1+a)/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×(1-x11-x12-x21)×((1+b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))×(1-r)/2 + x12×((1+a)/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x11×((1+k1×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + r×x12×((1+a)/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x21×((1+k2×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + x21×(1/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x11×((1+k1×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + r×x21×(1/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x12×(1/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + (1-x11-x12-x21)×(1/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x11×((1+k1×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))×(1-r)/2

x12’ = x11×((1+a)/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x12×(1/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + r×x11×((1+a)/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×(1-x11-x12-x21)×((1+b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + x12×((1+a)/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x11×((1+k1×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + x12×((1+a)/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x12×(1/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b))) + (1-r)×x12×((1+a)/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x21×((1+k2×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + x12×((1+a)/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×(1-x11-x12-x21)×((1+b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + x21×(1/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x12×(1/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))×(1-r)/2 + r×(1-x11-x12-x21)×(1/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x11×((1+k1×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + (1-x11-x12-x21)×(1/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x12×(1/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2

x21’=x11×((1+a)/(x11×(1+a)+x12×(1+a) + x21 + (1-x11-x12-x21)))×x21×((1+k2×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + r×x11×((1+a)/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×(1-

x11-x12-x21)×((1+b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + (1-r)×x12×((1+a)/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x21×((1+k2×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + x21×(1/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x11×((1+k1×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + x21×(1/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x12×(1/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))×(1-r)/2 + x21×(1/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x21×((1+k2×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b))) + x21×(1/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×(1-x11-x12-x21)×((1+b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + r×(1-x11-x12-x21)×(1/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x11×((1+k1×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2 + (1-x11-x12-x21)×(1/(x11×(1+a) + x12×(1+a) + x21 + (1-x11-x12-x21)))×x21×((1+k2×b)/(x11×(1 + k1×b) + x12 + x21×(1 + k2×b) + (1-x11-x12-x21)×(1+b)))/2

Eigenvalues of the Jacobian matrix for the system of iterative equations evaluated at the fixed point described above:

E1 = (2×(1×a^3 + 1×a^2×b + 2×a^3×b + 1×a×b^2 + 2×a^2×b^2 + 1×a^3×b^2 + 1×b^3 + 2×a×b^3 + 1×a^2×b^3))/(a + b + a×b)^3

E2 = 1/(a + b + a×b)^2×0.5×(2×a^2 + 4×a×b + 3×a^2×b + 2×b^2 + 3×a×b^2 + 1×a^2×b^2 + 1×a^2×b×k1 + 1×a×b^2×k1 + 1×a^2×b^2×k1 + 1×a^2×b×k2 + 1×a×b^2×k2 + 1×a^2×b^2×k2 - 3×a×b×r - 1×a^2×b×r - 1×b^2×r - 2×a×b^2×r - 1×a^2×b^2×r + 1×a×b×k1×r - 1×b^2×k1×r - 1×a×b^2×k1×r - 1×a×b×k2×r - 1×a^2×b×k2×r + 1×b^2×k2×r - 1×a^2×b^2×k2×r - ((-2×a^2 - 4×a×b - 3×a^2×b - 2×b^2 - 3×a×b^2 - 1×a^2×b^2 - 1×a^2×b×k1 - 1×a×b^2×k1 - 1×a^2×b^2×k1 - 1×a^2×b×k2 - 1×a×b^2×k2 - 1×a^2×b^2×k2 + 3×a×b×r + 1×a^2×b×r + 1×b^2×r + 2×a×b^2×r + 1×a^2×b^2×r - 1×a×b×k1×r + 1×b^2×k1×r + 1×a×b^2×k1×r + 1×a×b×k2×r + 1×a^2×b×k2×r - 1×b^2×k2×r + 1×a^2×b^2×k2×r)^2 - 4×(1×a^4 + 4×a^3×b + 3×a^4×b + 6×a^2×b^2 + 9×a^3×b^2 + 3×a^4×b^2 + 4×a×b^3 + 9×a^2×b^3 + 6×a^3×b^3 + 1×a^4×b^3 + 1×b^4 + 3×a×b^4 + 3×a^2×b^4 + 1×a^3×b^4 + 1×a^4×b×k1 + 3×a^3×b^2×k1 + 2×a^4×b^2×k1 + 3×a^2×b^3×k1 + 4×a^3×b^3×k1 + 1×a^4×b^3×k1 + 1×a×b^4×k1 + 2×a^2×b^4×k1 + 1×a^3×b^4×k1 + 1×a^4×b×k2 + 3×a^3×b^2×k2 + 3×a^4×b^2×k2 + 3×a^2×b^3×k2 + 6×a^3×b^3×k2 + 3×a^4×b^3×k2 + 1×a×b^4×k2 + 3×a^2×b^4×k2 + 3×a^3×b^4×k2 + 1×a^4×b^4×k2 + 1×a^4×b^2×k1×k2 + 2×a^3×b^3×k1×k2 + 2×a^4×b^3×k1×k2 + 1×a^2×b^4×k1×k2 + 2×a^3×b^4×k1×k2 + 1×a^4×b^4×k1×k2 - 5×a^3×b×r - 2×a^4×b×r - 7×a^2×b^2×r - 11×a^3×b^2×r - 4×a^4×b^2×r - 3×a×b^3×r - 8×a^2×b^3×r - 7×a^3×b^3×r - 2×a^4×b^3×r - 1×b^4×r - 3×a×b^4×r - 3×a^2×b^4×r - 1×a^3×b^4×r - 1×a^3×b×k1×r - 1×a^4×b×k1×r + 1×a^2×b^2×k1×r - 4×a^3×b^2×k1×r - 2×a^4×b^2×k1×r + 1×a×b^3×k1×r - 1×a^2×b^3×k1×r - 3×a^3×b^3×k1×r - 1×a^4×b^3×k1×r - 1×b^4×k1×r - 2×a×b^4×k1×r - 1×a^2×b^4×k1×r + 1×a^3×b×k2×r - 1×a^2×b^2×k2×r - 3×a^3×b^2×k2×r - 2×a^4×b^2×k2×r - 1×a×b^3×k2×r - 6×a^2×b^3×k2×r - 9×a^3×b^3×k2×r - 4×a^4×b^3×k2×r + 1×b^4×k2×r + 1×a×b^4×k2×r - 3×a^2×b^4×k2×r - 5×a^3×b^4×k2×r -

2×a^4×b^4×k2×r - 1×a^4×b^2×k1×k2×r - 2×a^3×b^3×k1×k2×r - 2×a^4×b^3×k1×k2×r - 1×a^2×b^4×k1×k2×r - 2×a^3×b^4×k1×k2×r - 1×a^4×b^4×k1×k2×r))^0.5)

E3 = 1/(a + b + a×b)^2×0.5×(2×a^2 + 4×a×b + 3×a^2×b + 2×b^2 + 3×a×b^2 + 1×a^2×b^2 + 1×a^2×b×k1 + 1×a×b^2×k1 + 1×a^2×b^2×k1 + 1×a^2×b×k2 + 1×a×b^2×k2 + 1×a^2×b^2×k2 - 3×a×b×r - 1×a^2×b×r - 1×b^2×r - 2×a×b^2×r - 1×a^2×b^2×r + 1×a×b×k1×r - 1×b^2×k1×r - 1×a×b^2×k1×r - 1×a×b×k2×r - 1×a^2×b×k2×r + 1×b^2×k2×r - 1×a^2×b^2×k2×r + ((-2×a^2 - 4×a×b - 3×a^2×b - 2×b^2 - 3×a×b^2 - 1×a^2×b^2 - 1×a^2×b×k1 - 1×a×b^2×k1 - 1×a^2×b^2×k1 - 1×a^2×b×k2 - 1×a×b^2×k2 - 1×a^2×b^2×k2 + 3×a×b×r + 1×a^2×b×r + 1×b^2×r + 2×a×b^2×r + 1×a^2×b^2×r - 1×a×b×k1×r + 1×b^2×k1×r + 1×a×b^2×k1×r + 1×a×b×k2×r + 1×a^2×b×k2×r - 1×b^2×k2×r + 1×a^2×b^2×k2×r)^2 - 4×(1×a^4 + 4×a^3×b + 3×a^4×b + 6×a^2×b^2 + 9×a^3×b^2 + 3×a^4×b^2 + 4×a×b^3 + 9×a^2×b^3 + 6×a^3×b^3 + 1×a^4×b^3 + 1×b^4 + 3×a×b^4 + 3×a^2×b^4 + 1×a^3×b^4 + 1×a^4×b×k1 + 3×a^3×b^2×k1 + 2×a^4×b^2×k1 + 3×a^2×b^3×k1 + 4×a^3×b^3×k1 + 1×a^4×b^3×k1 + 1×a×b^4×k1 + 2×a^2×b^4×k1 + 1×a^3×b^4×k1 + 1×a^4×b×k2 + 3×a^3×b^2×k2 + 3×a^4×b^2×k2 + 3×a^2×b^3×k2 + 6×a^3×b^3×k2 + 3×a^4×b^3×k2 + 1×a×b^4×k2 + 3×a^2×b^4×k2 + 3×a^3×b^4×k2 + 1×a^4×b^4×k2 + 1×a^4×b^2×k1×k2 + 2×a^3×b^3×k1×k2 + 2×a^4×b^3×k1×k2 + 1×a^2×b^4×k1×k2 + 2×a^3×b^4×k1×k2 + 1×a^4×b^4×k1×k2 - 5×a^3×b×r - 2×a^4×b×r - 7×a^2×b^2×r - 11×a^3×b^2×r - 4×a^4×b^2×r - 3×a×b^3×r - 8×a^2×b^3×r - 7×a^3×b^3×r - 2×a^4×b^3×r - 1×b^4×r - 3×a×b^4×r - 3×a^2×b^4×r - 1×a^3×b^4×r - 1×a^3×b×k1×r - 1×a^4×b×k1×r + 1×a^2×b^2×k1×r - 4×a^3×b^2×k1×r - 2×a^4×b^2×k1×r + 1×a×b^3×k1×r - 1×a^2×b^3×k1×r - 3×a^3×b^3×k1×r - 1×a^4×b^3×k1×r - 1×b^4×k1×r - 2×a×b^4×k1×r - 1×a^2×b^4×k1×r + 1×a^3×b×k2×r - 1×a^2×b^2×k2×r - 3×a^3×b^2×k2×r - 2×a^4×b^2×k2×r - 1×a×b^3×k2×r - 6×a^2×b^3×k2×r - 9×a^3×b^3×k2×r - 4×a^4×b^3×k2×r + 1×b^4×k2×r + 1×a×b^4×k2×r - 3×a^2×b^4×k2×r - 5×a^3×b^4×k2×r - 2×a^4×b^4×k2×r - 1×a^4×b^2×k1×k2×r - 2×a^3×b^3×k1×k2×r - 2×a^4×b^3×k1×k2×r - 1×a^2×b^4×k1×k2×r - 2×a^3×b^4×k1×k2×r - 1×a^4×b^4×k1×k2×r))^0.5)

Chapter 7

Conclusions

Among the many costs of sexual reproduction is the genomic conflict that ensues when the evolutionary interests of the sexes diverge (Parker, 1979). While sexual conflict can occur over a variety of different scenarios, research over the past several decades has resulted in the crystallization of all these diverse examples of sexual conflict into two distinct forms (Schenkel et al. 2018). On the one hand, Interlocus Sexual Conflict (IeSC) is usually modelled over the outcome of intersexual reproductive interactions (e.g., mating rates, copulation durations, sex allocation, parental investment, etc.), and is thought to be a result of traits that are sex-limited in their expression. On the other hand, Intralocus Sexual Conflict (IaSC) is modelled for traits expressed in both sexes but, subject to sexually antagonistic (SA) selection (Bonduriansky and Chenoweth 2009). While IaSC and IeSC have non-overlapping spheres of influence in their basic mathematical formalisms, there have been a growing number of arguments in favour of an interaction between the two (Pennell and Morrow 2013; Pennell et al. 2016). A major focus of this thesis was to investigate the potential interaction between IeSC and IaSC using the *Drosophila melanogaster* hemiclonal system (Abbott and Morrow 2011; Rice 1996). I began by asking whether changing the intensity of IeSC experimentally (by varying adult sex ratios), results in a change in the overall signal of IaSC at the level of fitness. Over the next three chapters, I measured the sex-specific genetic architecture, as well as the nature of sex-specific selection on a suit of sex-limited traits and traits that are shared between males and females. Finally, using a two-locus population genetic model, I investigated the resolution of IaSC as a consequence of sex-biased gene expression.

The major findings of this thesis were as follows:

1. Overall, experimentally increasing the intensity of IeSC resulted in a slight amelioration of the signal of IaSC. However, this trend was not statistically significant.

2. There were no genetic correlations between resistance related traits and persistence related traits. While male reproduction related traits were largely positively genetically correlated with female fitness, it was not immediately clear, whether this was driving the

interaction between IaSC and IeSC in my system. I also found that male pre- and post-copulatory traits were positively genetically correlated, and showed no evidence of trade-offs.

3. There was no statistically significant genetic correlation between male and female locomotory activity, ruling out the possibility that locomotory activity may be influencing patterns of IaSC. Nevertheless, there was some evidence that female activity was genetically correlated with SA fitness variation at female biased sex ratio. Interestingly, there appeared to be strong sexually concordant selection on faster development at male biased sex ratio.

4. I found wing shape to be a strongly sexually dimorphic multivariate trait, with males having wings with broader distal parts. There was substantial additive genetic variation as well as intersexual genetic correlation (r_{mf}) for various components of wing shape. Interestingly, r_{mf} was the weakest for the axis of wing shape variation where the sexes were best separated. I also found evidence of SA selection at male biased sex ratio, but not at the other two sex ratios, acting along a direction where there was little sexual dimorphism. I found that females with elongated wings, but males with shorter, stubbier wings enjoyed greater fitness at male biased sex ratio.

5. The results of my mathematical model suggested that the resolution of IaSC via modifier alleles bringing about sex-biased gene expression may not be as easy as previously thought. As long as modifier alleles affect the expression of the beneficial allele as well (in addition to affecting the expression of the deleterious allele), for a large set of values of selection coefficients and recombination rates, the resolution of IaSC is not automatically guaranteed.

Below I discuss some implications of these findings.

Interactions between IaSC and IeSC can be complex

There are several different ways in which IaSC and IeSC can interact, largely as a consequence of traits involved in IeSC not being fully sex-limited in their effects. First it is possible that resistance and persistence traits are positively genetically correlated. This would mean, strengthening IeSC in the population would also strengthen the degree of sexually concordant selection, leading to a weaker signal of IaSC. Second, it is possible that resistance and persistence traits have pleiotropic fitness effects when expressed in the

opposite sex. While there is no a priori expectation for these pleiotropic fitness effects to be positive or negative, Pennell et al. (2016) assumed that genes that code for resistance and persistence traits in females and males respectively exert a fitness cost when expressed in the opposite sex. This corresponds to a scenario where strengthening IeSC for one generation would lead to a stronger signal of IaSC. Lastly, if IaSC is primarily driven by reproduction related traits (Cox and Calsbeek 2009), strengthening IeSC could push males and females further away from their respective sex-specific fitness optima, strengthening IaSC as well. Thus, there is no universal expectation for the direction in which the interaction between IeSC and IaSC should proceed.

I found in my experiments that the direction of the interaction between IaSC and IeSC can be specific to the traits under investigation. For example, I showed that at stronger intensities of IeSC, there was stronger sexually concordant selection on development time, suggesting that stronger IeSC corresponds to a weaker signal of IaSC. On the other hand, I found evidence of statistically significant SA selection on wing shape, but only at higher intensities of IeSC, suggesting that IeSC and IaSC may reinforce each other. Overall, at the level of adult fitness, experimentally increasing the strength of IeSC led to a slight amelioration in the signal of IaSC in the population. These idiosyncrasies notwithstanding my results provide among the first experimental evidences of the interaction between IaSC and IeSC.

Evolving characteristics of the LH (or LH$_M$) populations – the role of laboratory adaptation

The LH population used in this thesis traces it ancestry to 400 wild *D. melanogaster* females sampled in 1991 from California, USA. Subsequently, the population has been maintained in laboratory conditions under fairly constant conditions and has been used to explore patterns of sexual conflict by a number of different groups over the last two decades. The findings of all these studies, including the results obtained in this thesis suggest that patterns of sexual antagonism in the LH population have evolved considerably over the last two decades (500-600 generations). These patterns point towards a trend, where IaSC in the LH population has evolved to become weaker. Some of the earlier studies (i.e., between 2000-2010) that employed the LH population to explore IaSC, found incredibly strong evidence of sexual antagonism. For example, Chippindale et al. (2001) and Gibson et al. (2002) reported strongly negative intersexual genetic correlations for adult

reproductive fitness for hemigenomes and X chromosomes sampled from the LH population, respectively. This pattern was also reported by Innocenti and Morrow (2010). Similarly, Pischedda and Chippindale (2006) reported a negative mother-son and father-daughter genetic correlation for fitness for haplotypes sampled from the LH population. Prasad et al. (2007) founded experimental evolution lines from the LH population and exposed them to selection only as males. This resulted in an increase in male fitness, but a reduction in female fitness, highlighting the presence of SA fitness variation in the ancestral LH population. In contrast, studies that attempted to measure signals of IaSC in the LH population subsequently did not find such unequivocal evidence for sexual antagonism. Collet et al. (2016) compared the intersexual genetic correlation for fitness ($r_{gw,mf}$) between two independent replicates of the LH population and reported that in one of the replicates $r_{gw,mf}$ was significantly less than 0. In the other it was positive but not significantly so, a finding also reported by Ruzicka et al. (2019). While Gibson et al. (2002) had reported substantial SA fitness variation on X chromosomes sampled from the LH population, Lund-Hansen et al. (2020) and Abbott et al. (2020) failed to detect unequivocal signs of X-linked sexual antagonism in the LH population. They subjected X chromosomes from the LH population to either female limited or male limited evolution, and found that an *increase* in the fitness of the selected sex, was not accompanied by a *decrease* in the fitness of the opposite sex. In continuation of this trend, in Chapter 3, I found a significantly *positive* $r_{gw,mf}$ at male biased, equal and female biased sex ratio. Taken together, these results point towards an unmistakable trend suggesting that IaSC in the LH population has declined in strength over the course of its evolution in the laboratory, particularly over the last decade or so. This trend is also reflected in the evolution of the degree of SA selection acting on individual traits during this period. For example, Long and Rice (2007) reported compelling evidence for IaSC over adult locomotory activity, while my results in Chapter 5a suggest that this conflict seems to have been resolved. Prasad et al. (2007) reported findings consistent with development time being associated with SA fitness variation. However, I found strong *sexually concordant* selection on development time. Abbott et al. (2010) reported strong sexual antagonism for wing shape along the axis of sexual dimorphism. On the other hand, I found no evidence of SA selection along the axis of sexual dimorphism in Chapter 5b, although there was IaSC along a different axis of wing shape variation.

Taken together, these results provide an interesting insight into the fact that signals of IaSC are not static, but can evolve within a span of a few hundred generations. There are a few

different ways in which this can happen. Using a variant of Fisher's Geometric Model, Connallon and Clark (2014) showed that as populations adapt to a novel environment, the degree of sexual antagonism should progressively increase. By contrast, mechanisms that resolve IaSC through the evolution of sex-specific genetic architecture (Connallon and Clark 2010; 2011; Day and Bonduriansky 2004; Spencer and Priest 2016) should actively promote the weakening of IaSC over time. Using some of these theories and other arguments, Duguay (2016) presented a comprehensive picture of how signals of IaSC are expected to evolve in conjunction with increasing adaptation to the laboratory environment. They argued that early on during the course of laboratory environment, populations are so maladapted to the novel environment of the laboratory that selection is predominantly sexually concordant. As populations begin adapting to it, however, most genotypes that are poor performing as both males and females go extinct, while the ones that are well-performing as males and females go to fixation. In this intermediate phase, genetic variation is largely sexually antagonistic, maintained by balancing selection in the two sexes. However, in the long run, given sufficient time most of the SA alleles eventually go to fixation, in spite of weak selection differentials. In this phase, again, sexually concordant fitness effects should dominate, yielding weaker signals of IaSC. According to this framework, the findings of my thesis such as significantly positive $r_{gw,mf}$, lack of IaSC over locomotory activity, strong sexually concordant selection on development time, suggest that the LH population is currently in the third phase of laboratory adaptation characterised by strong sexually concordant fitness effects.

Resolution of IaSC by modifiers bringing about sex-biased gene expression may not be straightforward

IaSC is generally thought to be resolved by the evolution of modifiers that bring about sex-biased gene expression (Connallon and Clark 2010; Day and Bonduriansky 2004). However, my analysis in Chapter 6 showed that this can only work when the modifiers modulate expression patterns at the SA locus in a very idealised way, i.e., in one of the sexes, they stop the expression of the deleterious allele, while leaving the expression of the beneficial allele *entirely* unaffected. I showed that if the modifiers reduce the expression of the beneficial allele as well, even if by a small magnitude, the invasion of the modifier, and the subsequent resolution of IaSC may not be automatically guaranteed. My results highlight that the molecular biology of gene expression regulation should not be overlooked while investigating the evolution of sex-biased gene expression.

Future directions

One of the shortcomings of my experiments was that they attempted to investigate how patterns of sexual antagonism plastically change upon subjecting a population to various sex ratio environments, *for a single generation.* They offered no insight into how patterns of sexual antagonism are expected to *evolve* upon exposing the population to these sex ratio treatments for a large number of generations. A large number of studies have experimentally evolved populations of insects at male biased or female biased sex ratios to investigate sexually antagonistic coevolution (Michalczyk et al. 2011; Nandy et al. 2013a; 2014; Wigby and Chapman 2004). It would be instructive to explore the evolution of the signals of IaSC in these populations.

The second shortcoming of my experiments is that the genetic variances and covariances I measured could be attributed to haplotypes consisting of the X chromosome, chromosome II, and chromosome III. My experiments did not allow me to partition effects between autosomes and X chromosomes. This is an important issue because mathematical theory predicts that X chromosomes and autosomes often differ in terms of their role in maintaining SA polymorphisms (Fry 2010; Ruzicka and Connallon 2020), resolution of IaSC (Connallon and Clark 2010), sex differences in trait variation (Reinhold and Engqvist 2013). A useful extension of my thesis would be to isolate a panel of X chromosomes and a panel of autosomes from the LH population (à la Griffin et al. 2016), and measure patterns of sexual antagonism at various sex ratios. Another important caveat of my thesis is that my experimental design completely ignored the contribution of the dot chromosome (chromosome IV). In fact, this is a shortcoming of all the studies that have used the *D. melanogaster* hemiclonal analysis system (Chippindale et al. 2001; Collet et al. 2016; Innocenti and Morrow 2010; Ruzicka and Connallon 2020). While the dot chromosome contains only a small portion of the *D. melanogaster* genome, it has had an interesting evolutionary history. Recent work has established that the dot chromosome was ancestrally an X chromosome that has now reverted back to being an autosome in *D. melanogaster* and other lineages (Vicoso and Bachtrog 2013; 2015). While discussing the role of X chromosomes at maintaining SA polymorphisms, Ruzicka and Connallon (2020) showed that the signal of sexual antagonism is by default stronger on X chromosomes (relative to autosomes) due to their asymmetric inheritance patterns, even when both autosomes and X chromosomes are equally enriched in SA polymorphisms. This issue raises an important question mark against empirical studies that reported an excess of SA variation on the X

chromosome (Gibson et al. 2002; Pischedda and Chippindale 2006). The *D. melanogaster* dot chromosome offers a way around this problem. It has an evolutionary history of having been an X chromosome. Second, it is unencumbered by methodological problems of the kind highlighted by Ruzicka and Connallon (2020) arising due to asymmetric inheritance patterns. Therefore, the dot chromosome can prove to be a good system to test the role of X chromosomes and autosomes vis-à-vis maintenance of SA polymorphisms.

An important caveat of the two-locus population genetic model I presented in Chapter 6 is that I restricted selection to haploid stage. This simplification has been used in the past (Kirkpatrick 1982). However, such models are applicable only to organisms with a prominent gametophytic stage. Furthermore, in the current model the influence of variation in dominance coefficients at the SA locus on the resolution of IaSC cannot be examined. Dominance coefficients are particularly important to sexual antagonism, because the fate of SA alleles is predicted to be highly contingent on the nature of their sex-specific dominance coefficients (Fry 2010; Jaquiéry et al. 2013). Therefore, it is imperative to extend the model described in Chapter 6 to a diploid system. Additionally, my model predicts that the evolution of sex-biased gene expression is inhibited by faster recombination rates. A convenient test of this prediction could be to measure the correlation between local recombination rates in various regions of the genome and the degree of sex-bias in the expression of genes located within those regions.

In conclusion, my thesis provides among the first experimental tests of whether IeSC and IaSC interact. I showed that the direction of this interaction can change depending on the traits under investigation. Under certain scenarios IeSC and IaSC can reinforce each other, while in certain other scenarios, strengthening one may lead to an amelioration of the other. Lastly, my theoretical results show that the resolution of IaSC through modifiers bringing about sex-specific gene expression may not be as easy as previously thought.

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